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## BIOLOGICAL ENIGMAS AND THE THEORY OF ENZYME ACTION

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### I

DURING the past twenty years the sciences which deal with inorganic physical phenomena have made astounding progress in the logical synthesis of their facts and theories.<sup>1</sup> The beginnings of this synthetic tendency lie, of course, in the middle part of the last century, in the work of such men as Faraday, Maxwell, and Mendeleeff. The discovery of radio-activity by Becquerel in 1896, and the demonstration by Thomson of the electron, in the following year, let loose the pent-up forces of an intellectual avalanche which swept scientific conservatism quite off its feet, and seems to be carrying our thought with thrilling rapidity towards a goal which metaphysical philosophers have for ages regarded with wistful longing. This goal is the comprehension of the physical universe in terms of a few simple conceptions.

The lines of demarcation which once were so rigidly drawn between the departments of physical science are disappearing before our eyes. The discovery of radio-activity, instead of adding a new science to the list, has

<sup>1</sup> D. F. Comstock and the present writer have attempted to give an elementary, but comprehensive presentation of the modern theory of matter in their book, "The Nature of Matter and Electricity," 1917.

brought us very close to a cancellation of all of the names except one; the demonstration of the existence of particles of negative electricity smaller than any known atom, instead of further complicating the facts of chemistry, has introduced a hundred simplifications. Mechanics, chemistry, optics, and the sciences of heat, electricity and magnetism are rapidly fusing into a single logical system, the ultimate terms of which are minute particles of positive and negative electricity, the ultimate laws those of electro-dynamics, and the ultimate problems those of the structures formed by these particles in space and of the changes which these structures undergo in time.

This startling progress in physics during the last two decades has not been the product of unadulterate empirical research. On the contrary, it has been made possible only by acts of daring speculation, which to certain more orthodox scientists of an earlier period might have seemed inexcusably foolhardy. However, their justification has often come so quickly and in such unequivocal terms, that methodological critics have been obliged to remain modestly silent. To indulge in a definite and detailed account of the structure and behavior of single atoms of hydrogen—particles far beyond the visual range of even the ultra-microscope—may seem no more a scientific undertaking than the fabrication of a fairy-tale; and yet when from this account there emerges by inevitable logic a mathematical formula corresponding exactly with the constitution of the complex spectrum of hydrogen, our minds are opened to the possibility that the speculation is pointing the way to a fundamental truth.<sup>2</sup> This impression becomes especially forcible when we consider that the constitution of this same hydrogen spectrum had for twenty-eight years defied the intellects of the best scientists, and by some had been regarded as incapable of explanation upon any simple hypothesis.

It is a fact of fundamental logical significance that the

<sup>2</sup> The reference is to the theory of N. Bohr, published in the *Philosophical Magazine*, 1913, 26; 1, 476 and 857.

progress represented by the modern electro-molecular conception of the physical universe has been achieved by the utilization of *a few general conceptions*, such as those of the electron and electrical action at a distance. These conceptions, although general, *i. e.*, universally applicable, are nevertheless extremely definite. They are also as tangible, or concrete, as it is possible to make them. It is nearly as characteristic of the modern theory of matter to eliminate abstractions as it is for it to gather up scattered facts and theories to unite them into an integral system. Although elements of abstraction still remain, they are reduced to a minimum by the increasing tendency to demand not only an algebraic symbol, but a visual picture of the processes of nature.

## II

It is perhaps not surprising that the astonishing progress of general physics during recent times should thus far have failed to exert any very notable influence upon the science of biology. From the point of view of the physicist, biological problems must be regarded as questions of special material structure, usually of a very intricate character, and involving the arrangement and history of units of matter for the most part larger than those upon which his attention is immediately concentrated. The program of modern physics is to build up the theory of all material structures by means of geometry and the dynamics of electrical particles. The first problem, logically, is that of the constitution of the atom, and as the solution of this problem is still unfinished, too much should not be expected of our knowledge of the configuration of particles and forces in higher aggregates of matter.

However, a critic who sees current events in the light of the history of science can hardly escape a twinge of disappointment at the rerudescence in biological theory, at the present time, of the doctrine of *vitalism*. The present, of all periods in the history of thought, is an hour of triumph of the monistic theory of nature, and yet now,

more frequently than during the nineteenth century, men eminent in biology seem to quail before the complexity and delicacy of the life process, and, while uttering mechanistic truths about life, to offer them as sacrifices to a spirit of vagueness and discouragement.<sup>3</sup>

It is my belief that this rejuvenation of mysticism and Aristotelian teleology is due not so much to a natural admiration on the part of biologists for obscure ways of thinking, as to their neglect of modern physics and of the methods of thought pursued in that science. It is the purpose of this paper, which is intentionally polemical in manner, to rebuke this tendency by commanding to the attention of biologists a general speculation concerning the life process, which—although incapable of immediate verification in all of its aspects—does answer the most perplexing questions raised by vitalism, and at the same time forms a perfectly distinct bond between biological theory and the modern theory of matter.

It is not improbable that the future will look back upon contemporary theoretical biology as a reactionary phase in the history of the science. The great synthetic energy of the Darwinian theory has been spent, has accomplished its magnificent results, but has left many tattered ends, by means of which a few of its enemies are attempting to tear down the entire structure once more. Even the remarkable discoveries which are classed under the name Mendelism are sometimes turned against the mechanistic conception of evolution. These discoveries, although patently of fundamental importance for the theory of life-processes, have as yet provided us with no new synthetic instruments of thought, but instead have generated an amazing and ever-growing list of abstract concepts. However, these concepts do furnish us with a means for the analysis of species in terms of their genetic determination and the recent studies of Morgan<sup>4</sup> and Goldschmidt<sup>5</sup> in

<sup>3</sup> Consider, for example, the contents of Haldane's recent address on "The New Physiology," *Science* (1916), N. S., 44, 620-632.

<sup>4</sup> Morgan, T. H., and others, "The Mechanism of Mendelian Heredity," 1915.

<sup>5</sup> See Goldschmidt, R., "Genetic Factors and Enzyme Reaction," *Science*

this field are pointing the way to synthetic considerations of far-reaching significance.

That biologists recognize the need of new light in the theory of heredity and of evolution, is clearly shown by the following quotations, from Bateson's Silliman lectures:

In spite . . . of the general attention devoted to the study of variation and the accumulation of material bearing on the problem, no satisfactory or searching classification of the phenomena is possible. The reason for this failure is that a real classification must presuppose knowledge of the chemistry and physics of living things which at present is quite beyond our reach. It is however becoming probable that if more knowledge of the chemical and physical structure of organisms is to be attained, the clue will be found through genetics, and thus that even in the uncoordinated accumulation of facts of variation we are providing the means of analysis applicable not only to them, but to the problems of normality also.

Again:

Somewhat as the philosophers of the seventeenth and eighteenth centuries were awaiting both a chemical and a mechanical discovery which should serve as a key to the problems of unorganized matter, so have biologists been awaiting two several clues. In Mendelian analysis we have now, it is true, something comparable with the clue of chemistry, but there is still little prospect of penetrating the obscurity which envelopes the mechanical aspect of our phenomena.

Again:

When with the thoughts suggested in the last chapter we contemplate the problem of evolution at large, the hope at the present time of constructing even a mental picture of that process grows weak almost to the point of vanishing. We are left wondering that so lately men in general, whether scientific or lay, were so easily satisfied. Our satisfaction, as we now see, was chiefly founded on ignorance.<sup>6</sup>

It will be perceived that the demand made by Bateson in these passages is not for new biological facts, but for physico-chemical conceptions in terms of which a chaos of biological facts, already at hand, can be explained, or systematized. Moreover, the emphasis is laid entirely upon the inability of the mind to conceive an explanation,

(1916), 43, 98-100. Also "Experimental Intersexuality and the Sex Problem," AMERICAN NATURALIST (1916), 50, 705-719.

<sup>6</sup> Bateson, W., "Problems of Genetics" (1913), 31, 32, and 97.

or a synthesis of these facts, rather than upon the necessity of detailed proof of some explanation which has already been offered. The contents of genetics would verify the proper conceptions if the human mind were only capable of suggesting them.

In another place,<sup>7</sup> Bateson says, with reference to the mechanism of cell division:

It is, I fear, a problem rather for the physicist than for the biologist. The sentiment may not be a popular one to utter before an assembly of biologists, but looking at the truth impersonally, I suspect that when at length minds of first rate analytical power are attracted to biological problems, some advance will be made of the kind which we are awaiting.

As a matter of fact, in the school of the physical chemists there has been in preparation, since the days of Thomas Graham, a system of knowledge which, even in its present unfinished form, has a most important and direct bearing upon mooted biological problems. This is the science of the *colloidal state*. The difficult abstractions and elaborate classificatory scheme, in terms of which the theory is now stated, will tend to be cleared up as our study of colloids comes definitely under the dominion of the general electro-molecular theory of matter. Intimate contact with the latter has already been established, indeed, through recent remarkable contributions by Langmuir,<sup>8</sup> dealing with the atomic constitution of solids and liquids. It is to colloidal chemistry that we must look for answers to the large majority of the fundamental problems of vital activity. These answers will be slow in appearing, however, if we refuse to look.

In fairness, it must of course be admitted that many biologists are keenly alive to the importance of the theory of matter, and especially of the theory of colloids, for the advancement of their science. However, possibly because the majority of these men are specialists in biochemistry, there seems to be a lack of coherent applications of mod-

<sup>7</sup> *Loc. cit.*, 41.

<sup>8</sup> Langmuir, I., "The Constitution and Fundamental Properties of Solids and Liquids," *Journal of the American Chemical Society* (1916), 38, 2221-2295; and other forthcoming papers in the same journal and in the *Physical Review*.

ern physico-chemical ideas to the problems of evolution and heredity, which make up the heart of the biological mystery.

It has for some years been my conviction that the conception of *enzyme action*, or of *specific catalysis*, provides a definite, general solution for all of the fundamental biological enigmas: the mysteries of the origin of living matter, of the source of variations, of the mechanism of heredity and ontogeny, and of general organic regulation.<sup>9</sup> In this conception I believe we can find a single, synthetic answer to many, if not all, of the broad, outstanding problems of theoretical biology. It is an answer, moreover, which links these great biological phenomena directly with molecular physics, and perfects the unity not alone of biology, but of the whole system of physical science, by suggesting that what we call life is fundamentally a product of catalytic laws acting in colloidal systems of matter throughout the long periods of geologic time. This view implies no absurd attempt to reduce every element of vital activity to enzyme action, but it does involve a reference of all such activity to some enzyme action, however distantly removed from present activity in time or space, as a necessary first cause. Catalysis is essentially a determinative relationship, and the *enzyme theory of life*, as a general biological hypothesis, would claim that all intra-vital or "hereditary" determination is, in the last analysis, catalytic.

The conception of enzyme action is, of course, one with which all biologists, including students of genetics, are extremely familiar.<sup>10</sup> Probably there is no student of morphogenesis who would not consider it absurd to deny that enzymes play a very important rôle in individual development. In a number of cases such participation has been clearly demonstrated by experiment, and the suggestion that the germ-cell contains "determiners" for

<sup>9</sup> See my two papers: "The Chemical Origin and Regulation of Life," *Monist* (1914), 22, 92-134; and "The Enzyme Theory of Life," *Cleveland Medical Journal* (1916), 15, 377 ff.

<sup>10</sup> On enzyme action in general, see Bayliss, W., "The Nature of Enzyme Action," 1914.

the production of enzymes, which, in turn, regulate certain aspects of the development, is a common one.<sup>11</sup> Several Mendelians have even hinted that the "unit characters" themselves are enzymes,<sup>12</sup> but so far as I am aware, no worker in genetics, with the exception of Goldschmidt, has regarded this conception as an important one.<sup>13</sup> Indeed, in the face of the nearly self-evident, they have turned away to vitalism and despair.

Consider, for example, the following quotation from Bateson.

We must not lose sight of the fact that though the factors operate by the production of enzymes, of bodies on which these enzymes can act, and of intermediary substances necessary to complete the enzyme action, yet these bodies themselves can scarcely be genetic factors, but consequences of their existence. What are the factors themselves? Whence do they come? How do they become integral parts of the organism? Whence, for example, came the power which is present in a White Leghorn of destroying—probably reducing—the pigment in its feathers?<sup>14</sup>

It is my contention in this and previous papers that statements of this sort can hardly represent anything less than intellectual blindness. On the supposition that the actual Mendelian factors are enzymes, nearly all of these general difficulties instantly vanish, and I am not acquainted with any evidence which is inconsistent with this supposition.

### III

Up to very recent times, although a great number of hypotheses to explain catalysis were in existence,<sup>15</sup> no

<sup>11</sup> See, for example, the following: Loeb, J., and Chamberlain, M. M., "An Attempt at a Physico-Chemical Explanation of Certain Groups of Fluctuating Variations," *Journal of Experimental Zoology* (1915), 19, 559–568. Moore, A. R., "On Mendelian Dominance," *Archiv für Entwicklungsmechanik* (1912), 34, 168–175. Riddle, O., "Our Knowledge of Melanin Color Formation and its Bearing on the Mendelian Description of Heredity," *Biological Bulletin* (1908), 16, 316 ff.

<sup>12</sup> See Bateson, "Mendel's Principles of Heredity," 1909, 268.

<sup>13</sup> The speedy publication of experimental results of great importance in this connection is promised by Goldschmidt. See above references.

<sup>14</sup> Bateson, "Problems of Genetics," 86.

<sup>15</sup> A comprehensive review of these theories and of the facts of catalysis and fermentation is given by Mellor, J. W., "Chemical Statics and Dynamics," 1914, 245–383.

completely satisfactory general theory of the process could be formulated. In this state of affairs, the use of the conception as a general explanatory agent in biology, could not be said to establish an unequivocal bond between biological regulation and the theory of matter. At the present day, however, it is possible to frame a hypothesis to account for catalytic action, which has general applicability and at the same time rests directly upon the ideas of modern molecular physics.

Ostwald defines a catalytic agent as "a substance which changes the velocity of a reaction without itself being changed by the process."<sup>16</sup> In the older terminology of the pioneer, Berzelius, it is "a substance which, merely by its presence and not through its affinity, has the power to render active affinities which are latent at ordinary temperatures."<sup>17</sup> According to Ostwald, catalytic power is a universal property of matter, for he says:

There is probably no kind of chemical reaction which cannot be influenced catalytically, and there is no substance, element, or compound which can not act as a catalyst.<sup>18</sup>

This being the case, it should often occur that a substance will catalyze a reaction which generates further quantities of the same substance, a process known as *autocatalysis*. Catalytic relationships may thus be classified into the autocatalytic and the *heterocatalytic*. I shall attempt to show that the former may be the more fundamental of the two relationships, and that reasons can be adduced for regarding autocatalytic power as a necessary property of every complex form of matter.

Perhaps the simplest illustration of a catalytic effect of any sort is that of the production of crystallization in a supersaturated solution of some substance by the introduction of a small crystal of the same substance. This of course has the form of an *autocatalytic* process. Although effects of this kind are included in Ostwald's classification of varieties of catalysis,<sup>19</sup> up to recent times

<sup>16</sup> Mellor, *loc. cit.*, 250.

<sup>17</sup> *Ibid.*, 246.

<sup>18</sup> *Ibid.*, 254.

<sup>19</sup> *Ibid.*, 255.

it might have been possible to raise a legitimate objection to the illustration on the ground that the induced change is not a chemical one. However, this objection is definitely disposed of by the recent work of the Braggs, and others, on the constitution of crystals,<sup>20</sup> which has shown that the unit of structure in solid bodies is usually the single atom, and not the molecule, since in crystals there is, as a rule, no exclusive arrangement of the atoms into molecular groups. The spacing of the atoms is such as to make it clear, moreover, that the forces which hold the total crystal system together are identical with those which we regard as underlying chemical affinity. In other words, in the crystal there is either no distinction between inter-atomic and inter-molecular forces (*i. e.*, between chemical affinity and cohesion), or else the entire crystal must be considered to be a huge polymeric molecule.<sup>21</sup> It is therefore perfectly legitimate to treat the process of crystallization as a chemical change, and to regard the initiation of this process under the conditions above described, as an example of autocatalysis, which may well be typical.

Although the results of crystal analysis indicate that no distinct molecules are to be found in the solid state, this is not true of the dissolved, or of the gaseous state. Moreover, on account of the fact that their component particles are held in place by forces of electrical attraction and repulsion, all molecules must possess their own fields of electrical force, and the field of any molecule must have a spatial form which is characteristic of that molecule. These field patterns will thus be different in the molecules of substances which differ chemically, and will be similar in molecules of the same or of an allied chemical substance.<sup>22</sup> The forces of cohesion in a crystal may be thought of as resulting from the fusion of a large number of these molecular fields into a continuous mosaic, and in such manner that their several axes are parallel.

<sup>20</sup> See Bragg, W. H., and W. L., "X-Rays and Crystal Structure," 1915.

<sup>21</sup> Cf. Langmuir, *loc. cit.*, 2221-2222.

<sup>22</sup> See Comstock and Troland, *loc. cit.*, 86-89.

However, such fusion can not fail to have an influence upon both the form and the strength of the fields in question, since it involves a redistribution of the atomic forces. This will take the form of an opening out, or expansion, which will necessarily reduce the coherence of the group of atoms originally forming the individual molecule. The degree of this "opening" of the field which occurs in crystallization must vary with the nature of the molecule, and is probably smaller for organic substances than it is for the majority of inorganic compounds.

The mechanism of the autocatalytic process of crystallization may be described somewhat as follows:

In a solution, or a gas, the molecules of the dissolved substance move about at random among the molecules of the solvent, and the orientation of the axes of their fields is entirely haphazard. However, as soon as a crystal of the solute is introduced, the field forces of the surface layer of atoms attract the dissolved molecules and at the same time tend to turn them on their axes so that, as they condense, they will fall into the pattern of the "space lattice" upon the plan of which the crystal is built.<sup>23</sup> As this is the most stable position which they can assume, they will tend to remain there and form a new surface layer of the crystal, to act in turn upon further molecules in the solution, until all of the surplus dissolved substance has been deposited.

The primary force bringing the molecules to the crystal face is of course not the surface field of attraction—surface tension field—but their temperature motion—or osmotic pressure. A similar force, of lower magnitude in the case of a supersaturated solution, is constantly disengaging molecules from the crystal and throwing them back into the solution. The action apparently ceases when the number of molecules deposited upon the crystal surface in unit time becomes reduced—owing to decreasing concentration—to an equality with the number leaving in the same interval.

<sup>23</sup> Cf. *ibid.*, 113.

The essential feature of the above described mechanism for the autocatalytic production of polymeric molecules may be illustrated to the eye by means of a model consisting of a board with a large number of small compass needles mounted upon it. If these needles are freed from the action of the terrestrial magnetic field and are then shaken into a random orientation, they may remain in this condition indefinitely. However, if a small number of adjacent needles be turned by some outside force so as to acquire a common direction, their combined magnetic fields will cause other neighboring needles to swing into line, so that the action must spread to all of the needles on the board. The field of an ideal compass needle has a simple bipolar pattern, and a symmetrical distribution of forces. In the cases of specific atoms and molecules, however, this is probably seldom true. Nevertheless, the general principles involved in their dynamic interaction would remain the same as those for the case of the compass needles.

It is clear that the explanation of autocatalysis above given accounts immediately only for the synthesis of polymeric molecules from individual units which are all alike. As a rule, chemical changes involve the interaction of different units, and it can easily be seen that the same general mechanism will apply to the catalysis of reactions of this sort as to that of simple crystallization. The principles involved in the process have been made especially clear in the recent articles of Langmuir.<sup>24</sup> Consider first a solution containing two kinds of molecules which can be deposited upon a crystal surface consisting of an orderly arrangement of these two molecular groupings in mosaic or lattice form. The second species of molecules may be considered, for example, to be those of the solvent, as in the case of "water of crystallization." There will be certain "elementary spaces"—as Langmuir calls them—upon the surface of the crystal, which will especially attract and orient the water molecules, and adjacent elementary spaces which will act in the same way upon the

<sup>24</sup> *Loc. cit.*, 2286-2292.

molecules of the solute. In this way the crystal or polymeric molecule will be built up out of two components by the simultaneous and parallel action of two initially combined species of molecular fields. This change is catalyzed by the crystal, and is an autocatalytic process involving the synthesis of two substances. It is clear that any number of substances may be influenced in this way by a similar, but more complex initial crystal form.

However, our explanation still remains somewhat special in its application, as in the majority of cases the products of catalysis do not adhere permanently to the catalytic surface. The extension of the explanation to cases of this sort is not difficult, since we have already seen that, even in the case of crystallization, the heat vibrations of the atoms are constantly throwing off molecular groups from the surface of the solid. As pointed out by Langmuir, the attraction between the surface and two molecular groups which have a strong affinity for each other may be less than the sum of the attractions of the surface for each of the groups, when separate.<sup>25</sup> This is due to the "closing up" or contraction of the fields of force of the groups as they come together. Hence combined groups of this sort will be more easily detached from the surface than will the uncombined groups, which will tend to be held in place until their mates fall into the right positions. The catalytic surface thus acts like an orienting sieve which on account of its special structure forces a chaotic crowd of individuals which come into contact with it, to fall into a special configuration. Many machines which accomplish exactly this effect are in use in the industries.

Thus far we have dealt only with the mechanism of autocatalysis. Heterocatalysis is probably to be regarded as an extension of the process of autocatalysis. It is obvious that exact similarity of the force patterns of the catalyzing and catalyzed systems is not essential. Indeed, the catalytic effect which is based upon direct similarity of structure between the two systems should be

<sup>25</sup> *Ibid.*, 2257, 2264-2266.

much weaker than that which accompanies certain types of structural *correspondence*, such as that existing between a body and its mirror-image, or between a lock and a key. Special structural relations of this sort probably exist between stereochemical isomers, between acids and bases, etc. It is easily conceivable that the patterns of certain surfaces may be capable of distorting other special configurations which come under their influence, so that they fall into new equilibrium figures, without these figures being of necessity identical with those of the catalytic system. The general principles of the action, however, remain the same.

Catalytic synthesis is a less common process in the laboratory than is destructive catalysis, but the laws of energy necessitate both effects, if either one is possible. Consequently the mechanism which we have described above must be an exactly reversible one, and must assist in the decomposition of molecular complexes as much as it aids in their synthesis. The deposition of the molecules to be decomposed, upon the catalytic surface would naturally follow the same principles as those stated for simple polymerization. In this state of deposition the field forces of the crystal surface would inevitably have a tendency to open up the field of the deposited molecule, thus rendering it more unstable than before, in which condition the temperature vibrations of the system could break it up more easily than in the undeposited state.

This weakening of the internal bonds of the molecule in the field of the catalytic surface corresponds with the weakening of forces of electrical attraction by increasing the "dielectric capacity" of the medium in which an electrical system is contained. It is the same action which permits water to dissociate neutral molecules into ions,<sup>26</sup> and is probably responsible for the high catalytic power of water, in general. However, in detail, the process must be a "personal" affair between individual water molecules and molecules of the dissolved substance, just as in the case of the crystal surface, since the ionizing effect

<sup>26</sup> Cf. Comstock and Troland, *loc. cit.*, 139-140.

of water does not appear to depend merely upon the chemical instability of the solute.

The increase in reaction velocity which characterizes catalysis is to be attributed to three more or less separable influences exerted by the catalytic surface, (1) the local increase in the concentrations of the reacting substances at the surface, (2) the impressment upon the attached molecules, of a relative orientation which is favorable to chemical union, or which in part constitutes such union, and (3) the spreading and weakening of the fields of force of the molecules, due to their interaction with the surface fields. The first factor, alone, would be of primary importance for the combination of free atoms—a relatively rare process—while the last factor, alone, would be responsible for the acceleration of simple decompositions. Reactions between two or more molecular groups, whether synthetic or metathetic, should be influenced by all three factors. Strutt<sup>27</sup> has shown that in certain typical chemical reactions, only one out of many millions of collisions between potentially reactable molecules results in chemical interaction. The active collisions probably coincide with the presence in the colliding system of favorable relative orientations and states of the molecular fields, which in the absence of a catalyst depend upon chance, but which in the presence of a catalyst are encouraged by the nature of the catalytic surface.

It is of course not possible in a paper of this sort to enter into the mathematics of the theory of catalysis which is outlined above.<sup>28</sup> Catalytic influence is obviously only one among many factors which affect a chemical reaction. Catalysis is possible only when the appropriate raw materials are provided, and when the energy relations of the system are such as to make the reaction thermodynamically conceivable. The heterocatalytic effect of a given substance may far outweigh its autocatalytic effect either

<sup>27</sup> Strutt, R. J., "Molecular Statistics of Some Chemical Actions," *Proceedings of the Royal Society* (1912), A, 87, 302-309.

<sup>28</sup> Cf. Mellor, *loc. cit.*, 250-254. Also Bayliss, *loc. cit.*, 49-71.

<sup>29</sup> For a development of the mass action relationships involved, see Langmuir, *loc. cit.*, 2287 ff.

because the energy changes do not favor the latter, or because in a given system the raw material for the auto-catalytic reaction is absent, while that for the hetero-catalytic reaction is present in abundance.

However, the above considerations would lead us to believe that all substances should show some tendency to form polymeric molecules or crystals. This appears to conflict with the classical division of substances into crystalloids and colloids, but this division, like all others, can not be expected to stand unmodified by the modern analysis. There is plenty of evidence from direct observation that many colloidal particles are simply very small crystals.<sup>30</sup> On the other hand, the molecules of polymeric substances of high molecular weight, such as starch and certain proteins, are probably of the same order of magnitude as small colloidal particles. From the point of view of the theory of matter, there is no fundamental difference between the general plan of a starch molecule and that of a crystal of sugar, and it is highly probable that the distinction between colloids and crystalloids rests upon purely quantitative relations, respecting the size of the polymeric structures (crystals) produced under ordinary conditions.

Large crystals are formed easily by simple substances whose molecules have open fields of force or highly unsaturated attractions. Small crystals are characteristic of more complex substances, common among the compounds of carbon, having relatively closed fields. Large mosaics of such molecules become unwieldy and are easily disrupted by the temperature vibrations. They are also built up more slowly than are mosaics of molecules with open fields. The distinction between these two classes of molecules is of course merely quantitative; no type of molecule has a completely closed field, and on the other hand no substance is capable of forming indefinitely large crystals in a finite length of time. The atomic structure of the solid phase of a colloidal gel is probably analogous

<sup>30</sup> See Ostwald, Wo., "A Handbook of Colloid-Chemistry," English translation, 1915, 56-66.

to that of the mass of small crystals, compacted together, which always results from the rapid crystallization of a supersaturated solution of a substance like, *e. g.*, sodium thiosulphate.

It is evident, then, that the general theory of catalysis which has been outlined is applicable to *enzyme action*, which almost certainly depends upon the deposition, or *adsorption* of the reacting substances upon the surfaces of colloidal particles.<sup>31</sup> Such adsorption, the molecular mechanism of which has been made very clear by Langmuir,<sup>32</sup> will tend to be specific, and the more specific the more complex is the structure of the units making up the mosaic of the surface. Molecules the field patterns of which fit closely into the fields of the surface will tend to displace others having a cruder correspondence. This follows from either electro-dynamics or thermodynamics, and obviously coincides with Fischer's classical conception of the lock and key relation between enzyme and substrate.<sup>33</sup>

It will be perceived that our theory of the catalytic process is simply a refinement and extension of the classical theory of "intermediate compounds," which has been proven true in so many instances.<sup>34</sup> "Adsorption compounds," which play the principal rôle in enzyme action, do not differ dynamically from chemical compounds in general, since the forces causing adsorption are the same as those responsible for chemical union. Conversely, catalytic action in which the catalyst is in a molecular or unpolymerized state will not necessarily differ in its mechanism from that characteristic of enzymes or of metallic surfaces.

#### IV

The suggestion that the fundamental life-process of growth is the expression of an autocatalytic chemical re-

<sup>31</sup> See Bayliss, *loc. cit.*, 104-123.

<sup>32</sup> *Loc. cit.*, 2267-2278.

<sup>33</sup> See Mellor, *loc. cit.*, 363.

<sup>34</sup> Cf., *e. g.*, Kendall, J., and Booge, J. E., "Studies on Catalysis. I. The Addition Compounds of Esters with Organic Acids," *Journal of the American Chemical Society* (1916), 38, 1712-1736.

action has been made independently by a number of investigators.<sup>35</sup> It will be perceived that on the basis of the foregoing theory of autocatalysis, this suggestion becomes closely allied to the familiar and ancient comparison of vital growth to the growth of a crystal. The customary objection to this comparison, viz., that a crystal grows by accretion whereas protoplasm increases by intussusception, loses its force as soon as we regard living matter as a complex mixture of substances suspended by colloidal subdivision in water, since there is no evidence that the individual colloidal particles do not grow by accretion. On the contrary, it is almost inconceivable that these bodies, which are the real chemical units of protoplasm, should grow in any other way. The growth of a system like a cell could be regarded as the resultant effect of a very large number of component growths, each governed by its specific autocatalytic mechanism. It has been shown by T. B. Robertson<sup>36</sup> that growth curves, with respect to the time, actually do coincide in general form with the curve characteristic of an autocatalytic reaction.

A multitude of observations substantiate the belief that the internal determination of cell-life rests primarily with the nucleus,<sup>37</sup> or with the chromatin substance of the cell, when no well-defined nucleus is present. Even in the highly organized cell, this substance can be seen to possess a mosaic structure, and it can be shown that for a given species this structure is sensibly constant,<sup>38</sup> so that it is necessary to suppose that a reduplication of chromatin units occurs with each cell-division. This process of reduplication is apparently made visible to us in mitosis.

<sup>35</sup> See, for example, Ostwald, W., "Ueber die zeitlichen Eigenschaften der Entwicklungsvorgänge," Vorträge und Aufsätze über Entwicklungsmechanik des Organismus, herausgegeben von W. Roux (1908), Heft 5, Leipzig.

<sup>36</sup> Robertson, T. B., "On the Normal Rate of Growth of an Individual and its Biochemical Significance," *Archiv für Entwicklungsmechanik* (1908), 25, 581-615; and subsequent articles in the same journal.

<sup>37</sup> See Wilson, E. B., "The Cell in Development and Inheritance," second edition, revised (1911), 30-31, 341-354.

<sup>38</sup> Cf. Boveri's *Individualitäts Hypothese* and "law of proportional nuclear growth."

The simplest hypothesis to account for such reproduction lies in the supposition that each unit can give rise to another unit substantially identical with itself.

The Weismannian theory of the constitution of the germ plasm,<sup>39</sup> which is typical of the so-called "corpuscular theories" of the life-process or of heredity, also demands the existence of vital elements, each possessing the power of reproduction *ad infinitum*. The general conceptions of this theory appear to find verification, first, in the facts already mentioned, and second, in the discoveries of Mendelism. The recent work of Morgan and his collaborators,<sup>40</sup> moreover, reveals clearly the intimate connection which exists between the corpuscular "unit characters" of Mendelian heredity and the histological units present in the chromosomes. Consequently, it would seem to be a fairly safe generalization, or at least an extremely probable hypothesis, which states that the distinctive properties of cells, tissues, and species are primarily determined by the nature of systems of colloidal particles contained in cell-nuclei and, originally, in some germ-cell nucleus.

However, in spite of the seeming strength of the evidence, some biologists are of the opinion that such a view as this must be rejected because it paralyzes thought. Consider, for example, the following quotation from Child.<sup>41</sup>

It is scarcely necessary to call attention to the fact that these [corpuscular] theories do not help us in any way to solve any of the fundamental problems of biology; they merely serve to place these problems beyond the reach of scientific investigation. The hypothetical units are themselves organisms with all the essential characteristics of the organisms that we know; they possess a definite constitution, they grow at the expense of nutritive material, they reproduce their kind. In other words, the problems of development, growth, reproduction, and inheritance exist for each of them, and the assumption of their existence brings us not a step nearer the solution of any of these problems. These theories are nothing more nor less than translations of the phenomena of life as we know them into terms of the activity of

<sup>39</sup> Weismann, A., "The Germ Plasm," English translation, 1893.

<sup>40</sup> *Loc. cit.*

<sup>41</sup> Child, C. M., "Senescence and Rejuvenescence," 1915, 11-12.

multitudes of invisible hypothetical organisms, and therefore contribute nothing in the way of real advance. No valid evidence for the existence of these units exists, but if their existence were to be demonstrated we might well despair of gaining any actual knowledge of life.

We have in this passage a clear statement of the essentiality of growth, as self-reduplication of specific substance, in the life-process. Consideration of Child's remarks will show that the difficulties which he raises are almost completely dissolved as soon as we postulate for the biological corpuscles the power of autocatalysis. In the light of our previous discussion, it can not be claimed that this is purely a verbal solution of the problem, as we have advanced definite reasons for believing that autocatalytic activity is a property of all chemical substances whatsoever, given the appropriate chemical environment. Since the environment of the chromatin particles has been made to order by evolution, the force of Child's criticisms would seem to be nil. Moreover, he certainly underestimates the importance of the facts which point to an actual corporeal determination of vital functions.

In view of this, it would appear advisable to accept the *Elementarorganismen*<sup>42</sup> as if they were clearly established facts, and proceed to consider what further light can be thrown upon biological problems by the conception of specific catalysis.

It is well known that in many cases, at least, the nucleus controls cell activity by liberating enzymes,<sup>43</sup> and the mass activity of cells in the form of specific tissues has been satisfactorily proven to depend, so far as it is directly chemical, upon the presence in these tissues of specific enzymes. In the field of general adult metabolism the determinative importance of catalysis would appear to be no longer a matter of debate. Analogy would lead us to believe that the same principle is of prime importance in the metabolism of development.

In laying emphasis upon the cardinal importance of the

<sup>42</sup> See Brücke, *Sitzungsbericht, Akademie der Wissenschaften, Wien*, (1861), 44, (2), 381-406.

<sup>43</sup> See Mann, G., "The Chemistry of the Proteids," 1906, 454 ff. Also, Loeb, J., "The Dynamics of Living Matter," 1906, 7-29.

enzyme for organic regulation we must of course recognize that the exact effects produced by catalysis depend, at all stages of development, upon the manner of its co-operation with other physical principles, which may involve the functioning of molar structures already present. However, as we retrace the course of ontogeny and of the evolution of any specific germ-cell, we should find that the number and importance of such structures decrease, although the construction of any given tissue-form always depends upon the action of specific enzymes in conjunction with preexisting tissue structures. It is not to be doubted for an instant that important preestablished structures exist even in germ-cells, and enter into the determination of their activity. It is therefore unfair to demand a catalytic explanation of such a complex process as karyokinesis, which shall not take into consideration the history or evolution of the cell.

The task of elucidating the exact mechanism by means of which vital regulation is maintained, and especially of showing how, in accordance with recognized principles of physics, a complex of specific, autocatalytic, colloidal particles in the germ-cell can engineer the construction of a vertebrate organism, is truly so formidable that it is unkind for the vitalist arbitrarily to deny us the use of any of these recognized principles. For example, we must be permitted to suppose that a large number of variables can unite in the production of a single effect. The greater part of the modern vitalistic worry over "organization" and vital "equilibrium"<sup>44</sup> appears to depend upon a tacit assumption, either that physical laws are not reliable, or that it is impossible for a number of variables to control simultaneously a single process. Both of these assumptions are self-evidently counter to the most fundamental presuppositions of physical analysis.

Although the fundamental life-property of the chromatin units is that of autocatalysis, it is necessary and legitimate to suppose that the majority of them sustain specific heterocatalytic relationships to reactions oe-

<sup>44</sup> Consider, for example, Haldane, *loc. cit.*

curring in living matter. This is because nuclear material makes up a relatively small percentage of protoplasm, and because the reactions governed by enzymes are ordinarily heterocatalytic.

It is a remarkable fact that the chemistry of the cell-nucleus has reached a stage of advancement superior to that attained by the chemistry of the cytoplasm. It appears that the essential constituent of chromatin is a substance called nuclein, which is composed of a basic, protein factor and nucleic acid. The facts indicate that the acid factor is the permanent and essential component of the nucleus, and organic chemical analysis seems to prove that only one kind of nucleic acid exists in animal tissues, although a different variety is to be found in the cells of plants.<sup>45</sup> If, as now seems probable, the genetic enzymes must be identified with the nucleic acids, we shall be forced to suppose that these substances, although homogeneous—in animal or plant—from the point of view of ordinary chemical analysis, are actually built up in the living chromatin, into highly differentiated colloidal, and colloidal-molar, structures. The apparent homogeneity results from the fact that ordinary chemical analysis provides us only with the *statistics of the fundamental radicles* which are involved.

To some minds, the idea that a portion of matter as small as a germ-cell can contain sufficient catalytic substance to control the destinies of a complex organism, seems hardly plausible. However, considering the slowness of such processes as growth, it is clear that the quantity of catalyst required will usually be smaller than that used in laboratory experiments; and it is a truism in chemistry that radical alterations of reaction velocities can be caused by the presence of almost infinitesimal amounts of catalytic material.<sup>46</sup> From the nature of the process, it is evident that only a few molecules of substance will be required to furnish the basis for an autocatalytic reaction which may eventually result in the pro-

<sup>45</sup> See Jones, W., "The Nucleic Acids," 1914.

<sup>46</sup> See Mellor, *loc. cit.*, 248-249.

duction of any desired amount of this substance; and a simple calculation shows that the chromatin of the human zygote has sufficient volume to contain about one quadrillion ( $10^{15}$ ) molecules the size of that of oxygen.<sup>47</sup>

In order that the enzymes of the germ-cell should be able to determine the form of the mature organism, they must have the power to govern (1) the physical and chemical properties of specific tissue material, (2) the position of specific tissues, (3) the size of these tissues and (4) their form. Since the physical properties of any piece of matter depend upon its chemical constitution, and since any chemical change can be regulated by catalysis, the mere presence of a specific catalyst in a favorable mixture is sufficient to determine the production of matter of any possible variety, in any possible amount. It is always necessary to assume that the history of an organic system is such as to have provided it with the raw materials necessary to its activities. If this is not the case, the system naturally perishes of "starvation."

The most primitive form of cell-division involves nothing more than reduplication, and this is the law of multiplication of the germ-plasm. Driesch<sup>48</sup> argues that to explain the reproduction of a nuclear "machine" which determines development, we must postulate another machine to carry out the operation, and so on *ad infinitum*. The nature of the autocatalytic process, however, shows that this conclusion is in error, since pure autocatalysis would tend to bring about an exact qualitative reproduction of any given plane or linear mosaic of specific units. In a nutritive medium such a mosaic would tend to grow in all of its parts by the deposition of similar substance. Primitive nuclear division (as, *e. g.*, in the Protista) may depend solely upon the physical instability of colloidal particles greater than a certain size, but it can hardly be

<sup>47</sup> This calculation is based on the following assumptions: (1) that the diameter of the germ-cell nucleus is .05 mm., and (2) that the molecules fill only one-sixth of the total volume of the nucleus.

<sup>48</sup> Driesch, H., "The Science and Philosophy of the Organism," 1908, 2, 341.

doubted that the complex mechanism of mitosis rests upon definite structural machinery, established by long periods of evolution.

In order to account for the differentiation of cell-nature which occurs in ontology, Weismann was led to assume a thoroughgoing differential segregation of the biophores of the original germ-cell in the course of embryological development; in other words, he supposed that in this process the rule of nuclear division is differentiation and not reduplication. This assumption, although undoubtedly a partial truth, is neither necessary nor in harmony with general biological probabilities, in the form in which it was made by Weismann.<sup>49</sup> Consequently the difficulties into which it has led his general theory, can be regarded as without important bearing upon the acceptability of corpuscular hypotheses at large.

Since reduplicating division is the established rule among unicellular organisms—which must have had a long evolutionary history—we should expect this rule to be conserved as far as possible in multicellular evolution. According to the general law of recapitulation, this should be especially true for the primary stages of ontogeny, for which Driesch's principle of the “equipotential system” appears often to hold. The blastula may well be simply an undifferentiated mass of germ-cells, analogous to a homogeneous colony of unicellular forms. Rudimentary differentiation may be brought about and determined by specific enzyme constitution, without differential partitionment of enzymes in segmentation, since the forces acting upon any cell must depend upon its *position* in the mass, and the activation or inhibition of a given enzyme may be conditioned by the presence of definite stimuli in definite intensities. This being the case, any cell could assume germinal characters if isolated from the total mass.<sup>50</sup>

<sup>49</sup> Weismann's theory, it must be recognized, assumes “doubling division” for the early stages of segmentation, a law which continues to hold for the “germ-tracks.”

<sup>50</sup> Cf. Hertwig, O., “Evolution or Epigenesis,” English translation, 1896.

However, the facts of "crossing over" observed in recent studies on the relation between Mendelian characters and chromosome constitution<sup>51</sup> show that the latter is not inviolate, even in purely germinal segmentation. Since the power possessed by cells to assume germinal character, even to the limited degree of being able to regenerate a single organ or tissue, seems to vary in inverse proportion to the degree of specialization of the cells, it is reasonable to suppose that Weismann's principle of differentiating division actually does operate in the higher stages of development. However, at no stage is it the only mechanism of differentiation, and it certainly is not the primitive means.

It is possible that cancer represents a return of tissue cells to a germinal or semi-germinal stage, due to the failure of a "stimulus of differentiation" to remain effective.

The control by the *genetic enzymes* of the position, size and form of specific tissues must involve, first, a quantitative regulation of the process of differentiation, which can be effected by the establishment of definite relations between the chemical constants of the catalytic reactions and the conditions under which the course of development necessarily places them; and, second, control of the planes of segmentation of the cells. To attempt a specification of the exact process by which this latter factor can be governed by the chemical constitution of the cell-nucleus, lies beyond the scope of the present paper, but it should be pointed out that in the last analysis chemical constitution means nothing but a definite spatial arrangement of electrical forces, so that there is nothing paradoxical in the determination of "pure form" by chemical agents.

As is evident in the quotations made above from Bateson, the dominant problem in the modern discussion of evolution is that of *the origin of variations*. It is the failure of Neo-Darwinians to explain the appearance of variations, and especially of new unit characters, which has led such writers as Driesch, Korschinsky and Wolff

<sup>51</sup> See Morgan, *loc. cit.*,

to speak of the "episode of Darwinism" and of *Das Sterbelager des Darwinismus*.<sup>52</sup> The enzyme theory of vital determination brings new life to the doctrine of evolution by accidental variation and natural selection, first, by showing that all fundamental variations should be discontinuous, or heterogenetic, as demanded by the mutation theory of De Vries, and second by revealing the exact mechanism of the production of these variations. The discontinuity follows from the existence of qualitative gaps between all specific chemical substances, such as those making up the system of genetic enzymes. The mechanism of production of variations is simply that of the initial production of any new chemical individual, *i. e.*, the fortuitous encounter of the appropriate molecules with the right relative orientations and at the correct speeds (*vide supra*). The "chance" nature of variation thus is made to depend upon that "molecular chaos" which is so very familiar to all physicists, but the implications of which for biology have thus far been largely neglected.

A moment's thought will show that, on the basis of the enzyme theory, variation should be *additive*, since an autocatalytic individual, once established, will tend automatically to maintain itself. The complete elimination of such individuals will occur only through the destruction of the entire germinal mosaic of which they form a part, an effect accomplished by natural selection unless the new enzyme is in harmony with functions which preserve the organism. It is very important to bear in mind that the catalytic complex which is supposed to underlie organic development and regulation has been determined in its nature by excessively exhaustive practical tests and, as a complex, by nothing else. It is therefore not surprising that the practical delicacy of the regulation which it actually subserves should be very great.

In other papers, I have discussed somewhat in detail the bearing of the enzyme theory upon the problem of the *origin of life*. On the basis provided by this theory, the

<sup>52</sup> See Kellogg, V. L., "Darwinism To-day," 1907.

origin of life can not be regarded as a catastrophic event; life depends upon an organized complex of selected catalytic material, and hence *some* life originates with each new, successful mutation. Of course, if we trace the process of the evolution of any given species back sufficiently far, we must eventually come to the *first mutation*, which would consist in the molecular production of an autocatalytic particle sustaining relations with its environment such as to make possible its continued growth and reproduction. I have used the name *protase* to stand for the "first enzyme" of the archebiotic process, but there is no particular reason for supposing that there was only one enzyme to which this name could apply.

There is considerable evidence that free autocatalytic enzymes exist in our biological universe even at the present day. Such an hypothesis would serve to account for the specific contagious diseases, such as measles, rabies, and smallpox, which have been demonstrated to possess "filterable viruses." The so-called Chlamydozoa probably fall in this class.

That the Chlamydozoa consist of free chromatin material is suggested by the late Professor Minchin, in his admirable paper on the evolution of the cell,<sup>53</sup> with the main outlines of which the enzyme theory would entirely agree. The single cell, and so-called simple protoplasm, must be regarded as the products of a detailed process of evolution, and hence can not form the ultimate explanatory units in biology. Next to the free autocatalytic particle, the simplest typical life-structure would consist of a single particle of this sort surrounded by an envelope of semi-liquid and chemically homogeneous substance with which it sustains a heterocatalytic relationship. The most primitive substance of this kind might be called *eoplasm*, to distinguish it from complex protoplasm, and the physical system made up of protase and eoplasm would represent a living cell in its most reduced form.

Minchin says, in the article referred to:<sup>54</sup>

<sup>53</sup> Minchin, E. A., "The Evolution of the Cell," AMERICAN NATURALIST (1916), 50, 5-39, 106-119.

<sup>54</sup> Loc. cit., 35-36.

The biochemist renders inestimable services in elucidating the chemical mechanisms of living organisms but the problem of individuality and specific behavior, as manifested by living things, is beyond the scope of his science, at least at present. Such problems are essentially of distinctive vital nature and their treatment can not be brought satisfactorily into relation at the present time with the physico-chemical interactions of the substances composing the living body. It may be that this is but a temporary limitation of human knowledge prevailing in a certain historical epoch, and that in the future the chemist will be able to correlate the individuality of living beings with their chemio-physical properties, and so explain to us how living beings first came into existence; how, that is to say, a combination of chemical substances, each owing its characteristic properties to a definite molecular composition, can produce a living individual in which specific properties are associated with matter in a state of flux.

It is my contention that the enzyme theory of life satisfactorily meets these general requirements.

## V

To arrive at a proper estimate of the importance of a general theory such as the one discussed in the present paper, necessitates considerable reflection. The path of scientific progress is beset by the pitfalls of conservative empiricism, on the one hand, and by those of radical speculation, on the other. To the radicals the enzyme theory presents an aspect of *a priori* self-evidence; to the conservatives it seems to be a vague generalization with no particular or specific facts to support it, approximately on the same plane as the statement that "life is motion," which Driesch says is about as useful as the proposition that "Kant was a vertebrate." Regarding the general enzyme theory, the following opinion has been expressed to me privately by an eminent zoologist.

The idea . . . is a perfectly familiar one. The trouble comes when we attempt to make a specific application of this idea to a concrete problem, which is what science demands if a pure speculation is to become a valuable working hypothesis. For instance, how an auto-catalytic molecule could produce the phenomena seen in the division of the chromosomes in the cell is by no means clear; nor is it clear why such molecules brought together during fertilization separate from each other at the maturation division. It is these specific questions that must first be answered, I think, before we can make much advance in

regard to the nature of the phenomena. . . . Of course, I realize that general ideas are always important for the development of science, only I think they should be advanced with caution and all attempts to make them appear as specific explanations should be avoided.

There are a number of principles of scientific methodology which have a bearing upon criticisms of this sort. In the first place such a statement as that "life is motion" would have value at a time when the connection between these two ideas had not been noted, or had been underemphasized to such an extent that eminent scientists were bemoaning the inability of the human mind to account for properties of life which the most superficial examination would show to be identical with those of motion. From my own point of view the proposition that "life is determined by specific catalysis" appears to be somewhat of the nature of a truism, indeed so much so that even if we had no direct evidence for the existence of enzyme action, we should be forced to invent the conception to account for the most general properties of living systems. It seems to me that this is exactly what a considerable number of biologists actually have done, and the only important error in their thinking lies in the application to the concept of such names as "biophore," "determinant," "unit character," "formative factor," "*Elementarorganism*," "*elan vital*," or "entelechy." This error, however, is fatal to progress, as it multiplies terminology and delays the synthesis of actual ideas which is the goal of scientific endeavor.

Furthermore, it is not true that the establishment of a general principle necessitates an examination of all of the concrete details of specific systems. If this were so, none of the fundamental laws of mechanics, such as the first and second laws of energetics or Hamilton's principle, would possess any rigidity, since they are derived from a study of what may be called the "entrance and exit" properties of mechanical systems, without reference to their exact contents. It is to be admitted, of course, that we can not rest content with this kind of knowledge, and that principles of this sort receive complete elucidation only when they are applied to specific systems.

tion only when the details of all systems are made clear, but the security of the principles themselves is affected scarcely at all by this analysis.

I do not claim that the enzyme theory of life possesses a general basis as adequate, for example, as that of the principle of least action. I do claim, however, that this is because the latter can be stated in terms of an exact mathematical formula, whereas the enzyme theory has to be given a qualitative description. The enzyme doctrine is supported at the present time by a considerable number of specific facts of cell chemistry, but it possesses a far more substantial bulwark in the general facts of vital function. Shall we deny that these facts are adequately established, or that they are important, or that they merit explanation? Shall we reject a definite physico-chemical conception which at one stroke explains the majority of the mass relationships of living matter, on the ground that the details of some special life-processes have not yet been described in terms of this conception? Or is it preferable to preserve the inexplication of these same generalities to furnish a basis for vitalism?

There are an indefinitely large number of ways in which the principle of the conservation of energy can be exemplified in special pieces of machinery, and there are just as many ways in which the principle of specific catalysis can operate. Instead of holding the energy principle in abeyance until we have seen how the action of a special mechanical system can be explained in terms of it, we usually assume it to be true, and shortly find the action in question very easy to understand. This would seem to be the only feasible method for employing any theoretical proposition, even if it is merely a novel working hypothesis. The trouble which arises in the attempt to apply the enzyme theory to specific problems is a normal result of the inertia of the human imagination, which does not immediately outline a plan for a machine to accomplish a definite purpose, even when it is provided with all of the principles of mechanics. Surely, however, the plan can never be developed if such principles are neglected.

## MUTATION IN DIDINIUM NASUTUM

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THE origin of heritable variations or mutations constitutes one of the most fundamental problems of biology. It has long since been recognized that evolution depends upon such variations, and they have consequently been extensively studied by a considerable number of investigators, *e. g.*, Darwin, DeVries, Batson, Kammerer, Tower, Stockard, MacDougal, Jennings, Morgan, *et al.* These studies have resulted in the accumulation of a mass of facts of great importance, but the nature of the origin of the variations in question is still shrouded in mystery.

In a series of experiments on the effect of conjugation and encystment in *Didinium*, extending from April, 1910, to May, 1914, there suddenly appeared, in the latter part of July, 1912, a marked difference in the rate of fission in the progeny of a single individual. This difference appears to have been permanent, as the results presented herewith indicate. And it seems to show, in opposition to the conclusions reached by a considerable number of investigators, that variations in organisms reproducing asexually are at times heritable.

The difference in rate of fission mentioned was discovered in a group of five pure lines, all of which had been carried from the beginning of the experiment. These lines all originated from the same individual, and before the mutation occurred they had produced, without conjugating, an average of 721 generations; and without encysting, 197 generations. Throughout this entire period there was remarkably little difference in the rate of fission in the five lines. The total number of fissions produced by these lines during the 40 days immediately preceding the appearance of the mutation was respectively 164, 171,

168, 166, and 168. Thus it is obvious that in the ancestors of the mutants nothing in the nature of mutations in the rate of fission had occurred for many generations. This indicates that mutations do not ordinarily occur in asexual reproduction.

During the period of 40 days mentioned above, ending July 10, all of the lines were in excellent condition and not a single individual died. On July 12, however, one line died out and on the 14th three more died, leaving but one line. For several days preceding the temperature was very high. It was recorded twice daily and these records show that it reached a maximum on July 9, when it was  $28.5^{\circ}$  at 7:30 A.M. and  $31^{\circ}$  at 6 P.M. It was, however, high continuously from the 4th on, and during this period reproduction was exceedingly rapid, as Table I indicates. It was at the close of this period of rapid multiplication that the four lines mentioned above died out and it is probable that this extraordinary environmental condition had much to do with the nature of the variations in the progeny of the remaining line, although similar variations did not occur in four other groups of lines that were running parallel with the one under consideration.

From the remaining line mentioned above five new lines were started on July 15. For the first five days, the rate of fission in these lines was nearly the same, the total number per line being 26, 27, 28, 26 and 28, respectively. During the next five days the difference became somewhat greater, the total number of fissions per line being 21, 19, 18, 17 and 17, respectively. On the day following this period, the line which had produced 19 fissions died out and was replaced by a new line from the one which had produced 21. There were thus two lines having more rapidly, and three lines having less rapidly, dividing ancestors, all, however, originating from the same individual. On October 14 the lines in both groups were increased to five and thus they were continued until the close of the experiment, which extended through 315 days. The individuals in all of the lines in each group repro-

TABLE I

## THE RELATION BETWEEN THE FISSION-RATES OF TWO STRAINS OR GROUPS OF LINES ISOLATED FROM THE PROGENY OF A SINGLE DIDINIUM

June 1, 1912 to May 27, 1913

Each column under the brackets represents a line and each number in the column the total number of fissions for five days; *d* indicates that the line died out; *c*, that it encysted; the brackets show the ancestry of the new lines established. The ancestors of the first five lines had passed through 553 generations without conjugation when these lines were started. Not all the lines that died out are indicated in the table. Whenever a line died out more than once in a five-day period, as sometimes happened, it is recorded only once.

					Average Total No. Fissions for Five-day Periods	
					First Group of Lines	Second Group of Lines
<b>Pure Line with 553 ± Generations without Conjugation</b>						
23	23	22	21	22		
14	15	14	15	15		
17	18	18	17	17		
18	19	18	18	21		
20	21	21	21	20		
23	22	22	22	21		
21	22	21	21	23		
28	31	32	31	29		
Total 164	171	168	166	168		
30	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>		
26	27	28	26	28	26½	27½
21	19	18	17	17	20	17½
22	<i>d</i>	22	<i>d</i>	<i>d</i>	22	22
17	18	15	15	15	17½	15
20	20	15	16	16	20	15½
<i>d</i>	26	20	22	21	26	21
24	24	17	20	19	24	18½
25	26	18	24	19	25½	20½
20	21	13	18	15	20½	15½
<i>c</i>	19	15	16	15	19	15½
26	25	20	22	21	25½	21
25	24	19	19	20	24½	19½
17	17	14	15	14	17	14½
15	16	12	12	11	15½	11½
<i>d</i>	13	9	9	9	11½	9
7	7	3	5	3	7	3½
15	16	12	11	9	15½	10½
<i>d</i>	18	13	13	15	18	13½
8	8	9	10	8	8½	6
13	12	12	12	<i>c</i>	12½	8½

TABLE I (*continued*)

Pure Line with 553 ± Generations without Conjugation												Average Total No. Fissions for Five-day Periods	
												First Group of Lines	
<i>d</i>	10	11	9	<i>e</i>		8		8		7		Second Group of Lines	
<i>e</i>	12	12	12	12		9		9		<i>d</i>		12	9
12	12	13	13	13		<i>d</i>	8	8	9	9	12 $\frac{3}{5}$	8 $\frac{1}{2}$	
12	11	11	10	11		9	8	8	8	7	11	8	
9	10	10	10	10		<i>d</i>	5	6	7	6	9 $\frac{1}{3}$	6	
9	10	10	<i>d</i>	11		7	5	6	6	<i>d</i>	10	6	
10	10	<i>d</i>	8	9		5	3	5	<i>d</i>	5	9 $\frac{1}{4}$	4 $\frac{1}{2}$	
9	9	9	9	9		5	<i>d</i>	5	<i>d</i>	6	9	5 $\frac{1}{3}$	
10	10	10	<i>d</i>	8		7	5	7	6	6	9 $\frac{1}{2}$	6 $\frac{1}{5}$	
10	<i>e</i>	<i>d</i>	<i>d</i>	9		6	7	7	7	7	9 $\frac{1}{2}$	6 $\frac{1}{3}$	
10	11	12	11	10		8	9	8	8	8	10 $\frac{1}{3}$	8 $\frac{1}{5}$	
11	<i>e</i>	13	<i>e</i>	13		<i>e</i>	7	8	<i>d</i>	5	12 $\frac{1}{3}$	6 $\frac{2}{3}$	
11	12	12	12	12		8	<i>e</i>	6	8	4	11 $\frac{1}{3}$	6 $\frac{1}{3}$	
<i>d</i>	13	<i>d</i>	13	12		9	6	9	8	8	12 $\frac{2}{3}$	8	
<i>d</i>	14	14	14	14		10	12	10	10	10	14	10 $\frac{2}{3}$	
15	<i>d</i>	13	14	14		10	10	9	10	10	14	9 $\frac{1}{3}$	
12	12	12	13	12		9	8	8	8	8	12 $\frac{1}{3}$	8 $\frac{1}{3}$	
13	13	13	15	14		10	10	9	10	10	13 $\frac{3}{5}$	9 $\frac{1}{3}$	
12	11	11	<i>e</i>	12		9	9	8	9	8	11 $\frac{1}{3}$	8 $\frac{1}{3}$	
10	10	10	11	10		8	9	7	8	6	10 $\frac{1}{3}$	7 $\frac{1}{3}$	
11	11	11	11	11		8	8	8	8	7	11	7 $\frac{1}{3}$	
10	10	11	10	10		5	7	6	6	<i>d</i>	10 $\frac{1}{3}$	6	
8	11	11	11	10		8	8	6	7	8	10 $\frac{1}{3}$	7 $\frac{2}{3}$	
10	13	12	11	12		9	11	8	8	8	11 $\frac{1}{3}$	8 $\frac{1}{3}$	
11	14	<i>d</i>	<i>d</i>	<i>d</i>		10	6	11	11	10	12 $\frac{1}{2}$	9 $\frac{2}{3}$	
10	<i>d</i>	10	<i>d</i>	12	9	9	7	<i>d</i>	8	<i>d</i>	10 $\frac{1}{4}$	8	
7	8	9	9	8		7	7	7	7	7	8 $\frac{1}{3}$	7	
9	13	12	12	<i>d</i>		10	8	9	9	9	11 $\frac{1}{2}$	9	
7	12	13	13	11		10	8	8	9	8	11 $\frac{1}{3}$	8 $\frac{2}{3}$	
10	14	12	12	14		9	8	9	9	10	12 $\frac{1}{3}$	9	
8	12	12	11	12		9	7	8	9	7	11	8	
<i>d</i>	9	8	9	9		6	<i>d</i>	<i>d</i>	6	6	8 $\frac{1}{3}$	6	
10	11	<i>d</i>	11	11		9	7	6	<i>d</i>	6	10 $\frac{1}{2}$	7	
8	8	<i>d</i>	<i>d</i>	<i>d</i>		3	7	7	6	6	7 $\frac{1}{2}$	5 $\frac{1}{3}$	
9	9	7	<i>d</i>	<i>d</i>		7	<i>d</i>	7	7	7	8 $\frac{1}{3}$	7	

TABLE I (*concluded*)

Pure Line with 553 ± Generations without Conjugation												Average Total No. Fissions for Five-day Periods	
												First Group of Lines	Second Group of Lines
9	9	8	7	7	6	6	7	5	5	8	12	5½	8½
12	d	11	12	13	9	9	8	d	7				
7	9	10	9	9	6	5	3	d	7	8½	5½		
16	11	13	14	14	d	10	7	d	9	13½	8½		
d	7	7	8	6	4	3	5	4	4	7	4		
10	9	9	9	6	7	3	4	4	4	8½	4½		
10	8	11	7	9	8	7	8	7	d	9	7½		
d	8	10	d	10	6	d	8	5	d	9½	6½		
Total average generations per line in 315 days.....												838 ±	634 ±
Number of lines died out.....												30	33
Number of lines encysted.....												8	3
Number of times conjugation occurred before transfer was made.....												2	0

duced at practically the same rate, but those in the former, considerably more rapidly than those in the latter. During the 315 days each line in the one group produced approximately 838 generations,  $2\frac{2}{3}$  per day, and each line in the other group approximately 634 generations, 2 per day.

The averages for the five lines in each group for five-day periods are presented in Table I and plotted in Fig. 1. By referring to this table and the figure it will be seen that the difference in rate of fission in the two groups remained fairly constant throughout the entire 315 days, and that in both groups the rate was high in July and August, 1912, after which it decreased considerably and then remained fairly constant.

The fluctuations in rate of fission were closely associated with variations in temperature. This was true for twenty-four-hour periods as well as for the five-day periods given in the table. During July and August, when the fission rate was high, the temperature was in general much higher than it was during the rest of the time, when the fission rate was relatively low. At the

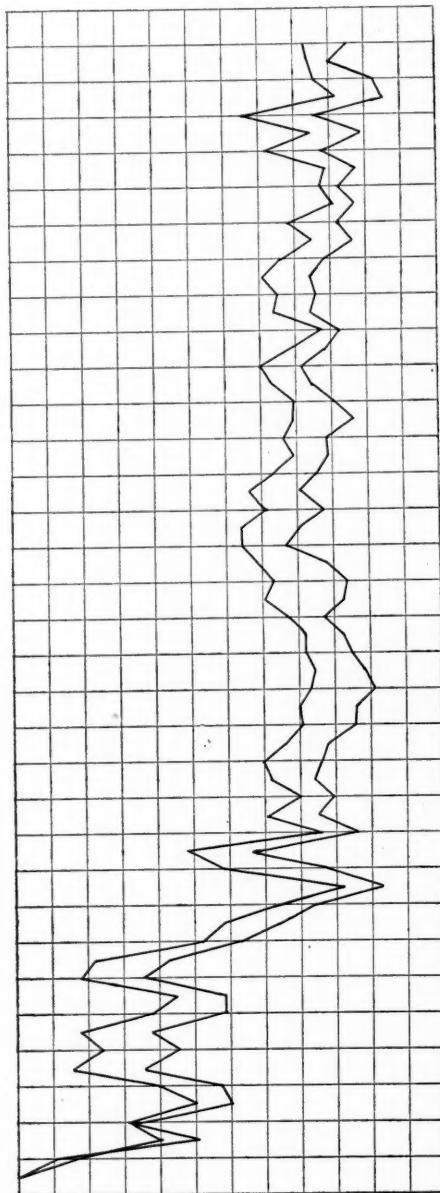


FIG. 1. Curves representing the fission rates in two groups of lines of *Didinium* originating from a single individual  $721 \pm$  generations after conjugation and  $197 \pm$  generations after encystment. Each point in the curves was obtained by averaging the rate of fission of all of the lines in each group for five successive days. Ordinates equal average daily rate of fission, abscissas days from the beginning of the experiment.

Note that the difference in the rate of fission in the two groups is essentially the same during the entire 315 days.

close of the experiment, however, the rate of fission was not as high as the temperature at this time would lead one to expect. The didinia in both groups appeared to be in poor condition. There were numerous very small individuals produced and an unusually large number of monsters. Conjugation was prevalent, but it was almost impossible to induce encystment. The death-rate was, however, not abnormally high. Whether or not the lines would have recovered from this depression if the experiment had been continued, is a question which can not be definitely answered.

Before the experiment was closed some cysts were secured in both groups. These were kept in a damp chamber as usual until the following year. Then they were put into culture fluids of various sorts containing paramecia; but only a few developed, all of which belonged to the more rapidly dividing lines. From these, five new lines were started and carried on for 40 days. During all this time the condition of the individuals was much as it had been immediately before encystment.

Throughout the entire experiment the didinia were cultivated in rectangular watch-glasses having a depression with a curved bottom. These dishes were piled one upon the other and kept in a damp chamber. All of the didinia were fed with paramecia from the same cultures. At each feeding an equal amount of solution was taken from two of the most vigorous of four pint cultures which were continuously kept in as flourishing conditions as possible by adding fresh water and a little timothy hay from time to time. The two equal quantities of solution were then thoroughly mixed and two drops of this mixture containing numerous paramecia were put into each of as many watch-glasses as there were didinia cultures. One drop of solution containing one didinium was then taken from each of the didinia cultures and added to each of the watch-glasses containing the paramecia. The remaining didinia, after recording the number of generations produced, were destroyed or used in studying conjugation.

and encystment. During the coldest weather it was sufficient to transfer every other day, but during the warmest weather it was found advantageous to transfer twice a day. Nothing was sterilized in these experiments, but the same pipet was used in all transfers and the watch-glasses not in use were exposed to the air and allowed to dry. Moreover, from time to time the didinia in each line in either group were transferred directly to the watch-glasses from which the didinia of the other group had just been taken. In these dishes there always remained considerable solution, in some instances a drop or more. Furthermore, in a few cases didinia from the more rapidly dividing lines were transferred directly without the addition of fresh food to dishes in which more slowly dividing lines had died or from which all of the didinia had been removed.

Such treatment had no appreciable effect on the relative rate of fission in the two races. It is obviously evident, therefore, that the difference in the rate observed was not due to difference in the bacterial contents of the solution if there really was any such difference, nor was it dependent upon selection, natural or otherwise, for members of the more rapidly dividing pairs were always transferred in all lines. And the number of lines lost by death and encystment was essentially the same in both. In the one 30 were lost by death and 8 by encystment, in the other 33 by death and 3 by encystment. Assuming that the weaker lines died out in every case, it is evident that in this respect both races were subjected to practically the same sort of selection. And since all of the cultures were subjected to the same conditions otherwise, it is clear that the difference in the rate of fission in the two races must have been due to the constitution of the organisms.

We have consequently demonstrated that marked variations in the rate of fission may appear quite suddenly in the progeny of a single individual without conjugation or encystment, that some of these variations are heritable, and that they can probably be produced by subjecting the individuals to abnormally high temperature.

By referring to the table it will be seen that the mutation investigated originated, as previously stated, at the close of a period of extraordinarily high rate of fission and immediately after a short period of very high death-rate in which all but one of the lines died out. At the beginning of this period and at the close of the preceding period the individuals were very small and showed all the characteristics in behavior common to individuals about to conjugate. Whether or not anything in the nature of a nuclear reorganization in preparation for conjugation occurred in these didinia is not known, but ordinarily such phenomena do not begin until some time after union takes place in conjugating specimens. Moreover, the period between fissions was not long enough to admit of much in the way of reorganization aside from what ordinarily occurs during the process of fission. Whether or not the ancestors of the mutants were actually homozygous is not known. If they were not the mutation may possibly have been due to a rearrangement of unit characters represented in the chromosomes during fission resulting in a change in dominance. However, if this did actually take place it is not in accord with the results obtained in very extensive investigations, all of which seem to show that changes in dominance do not occur in asexual reproduction. It is probable, therefore, that the mutation was due to a direct effect of the environment on the physiological processes in the organism and not to inherited nuclear phenomena largely independent of the immediate environment.

The mutation theory so ably championed by DeVries has of late lost greatly in prestige, owing largely to the contention that the plants (*Onothera*) in which DeVries discovered mutations were hybrids. If the conclusion reached in this work proves to be correct it will strongly support the theory in question. It will demonstrate that marked variations may appear suddenly in organisms reproducing asexually, that such variations may be heritable and that they may have a decided evolutionary value.

This conclusion, though in opposition to a great bulk of the experimental evidence gathered by some of the foremost biologists, Johannsen, Maupas, Morgan, Castle, Jennings and many others, is supported by some of the results obtained by Barber (1907), Calkins and Gregory (1913), Middleton (1915) and Jennings (1916).

#### SUMMARY

In a race of didinia originating from a single individual there suddenly appeared a heritable variation in the rate of fission. This variation occurred 721 generations after conjugation and 197 generations after encystment.

Two strains were isolated from this race and kept under observation for 315 days. During this time the lines in one strain produced an average of  $838 \pm$  generations ( $2\frac{2}{3}$  per day) and those of the other  $634 \pm$  generations (2 per day).

## THE METHOD OF EVOLUTION FROM THE VIEW- POINT OF A GENETICIST<sup>1</sup>

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A SYMPOSIUM upon the method of evolution, participated in by students from widely different provinces, can hardly be expected to develop harmony of opinion. But to be assigned a place in such a discussion as a representative of one field of endeavor does not imply that the conclusion one reaches will necessarily differ from that of his co-symposiasts; for the question of the method of evolution is a question of fact, and when the fact is discovered, it will be recognized as completely by the paleontologist as by the physiologist. And, in particular, to be assigned the final place in the argument does not mean that the doctrine preached will be, or even is, the last word on the subject. In the present state of knowledge of biology the most that can be expected of an address on this subject is a statement of principles which should guide us in a search for the facts. Beyond these principles there are justifiable suspicions, and there may even be militant conjecture, but little else.

The first fundamental principle for the guidance of one who would find the method of evolution is a principle common to all sciences which seek to explain the occurrences of a remote past. No agencies may be assumed to have operated fifty million years ago of a different order from those that operate to-day. If the phenomena of the present afford a plausible, or even possible, explanation of the past, there must be no appeal to other phenomena, the like of which do not now exist. Just as a geologist mentally constructs the rock strata a thousand feet below the surface, and the glacial drift of regions

<sup>1</sup> Concluding paper of a symposium on the method of evolution before the zoological section of the Michigan Academy of Science, March 28, 1917.

now temperate, on the basis of processes now going on in certain parts of the earth; and just as the astronomer creates the planetary systems in his mind by forces that still govern, so the biologist must conceive evolution in the past to have been the result of agencies that are still causing change to-day. Whatever causes evolution now may conceivably have caused it during the early history of living things, and there are no circumstances which compel one to devise other causes for past change.

Adherence to this principle automatically removes the first solution of the problem of the method of evolution from the realm of the investigator who deals only with past events or the results of past occurrences, and places it in the hands of him who studies present-day phenomena. Conclusions based on statistics have repeatedly shown how dangerous it is to argue from end results to causes. The compiler who finds that among the poorer classes of a population the ratio of male children to female is higher than in the well-to-do classes, and concludes that deficient nutrition causes the high male-production, might also have discovered, had his investigations borne upon that point, that the poorer classes lived in houses protected with a cheaper grade of paint or even without this protection. It would have been ridiculous to conclude that cheap paint favored boys, but that conclusion would have been as nearly *proven* by the data collected as was the more plausible conclusion involving nutrition. Causes are not safely to be judged from results. In discovering the method of evolution, the initiative is denied the paleontologist, zoogeographer and the morphologist. No doctrine of scientific cloture is here advocated, however, for the right of debate and even of veto is still theirs. The experimentalist alone may propose, but his colleagues employing the older forms of investigation may, and doubtless will, dispose. The experimentalist accepts his burden cheerfully. He knows that he may be unable to create a correct theory, but he prefers dispensing with a theory to adopting the wrong one.

If you grant that evolution in the past was caused by the same agents as cause evolution to-day, to what phenomena of living things will you apply this principle? Evolution requires two things, namely, modification and inheritance. Given these two things, the occurrence of a new characteristic and the inheritance (or even only the heritability) of the new characteristic, evolution has occurred. It matters little now what becomes of the individual or individuals possessing a new heritable character. They may even perish before they leave offspring, yet evolution has occurred. What became of these incipient races was the theme of the evolutionists of the past half century, who devised many and fanciful theories to account for their preservation or their destruction. To-day we are concerned less with the fitness of the new characteristic for the environment; we demand rather to know how the new feature arose and why it was inherited.

Fortunately there is no fundamental disagreement with regard to inheritance. Too much is known of the mechanism of inheritance to allow of dispute. The chromosomes have been saddled with the main responsibility. There has never been any general attempt to refer inheritance to the environment. No one has supposed that a goose egg laid in the sand would produce a turtle. It is true, the cytoplasm has a share in determining what shall develop from an egg; so does oxygen, and so do other components of the medium, as can be readily shown by altering those components. What develops in the presence of this cytoplasm, and out of this cytoplasm, depends specifically, however, upon the chromosomes. Disputes regarding this fact have seldom been dragged into arguments over the method of evolution.

With the primary requirement of evolution, the production of new characters, matters have been otherwise. The mode of origin of modifications has not shared the good fortune of the mechanism of heredity. There is no need to cite the hosts of opinions that have been held by reputable scientists regarding the inception of evolu-

tionary change. They have ranged from those who would make the living world of to-day wholly the product of the environment, to those that deny any participation of external factors in the course of evolution. Where in this array of opinion is the probable truth? To answer this question will be to express another opinion; but it is possible to formulate an opinion which is based upon principle, and which will therefore be more inviting than mere conjecture.

With the aid of Sir Charles Lyell, who more than any one else has taught us to seek the explanation of past events in present processes, let us look about us for the cause of diversity among individuals. We may ignore differences which, from their fundamental nature, are not permanent, that is, modifications which are not heritable; for of such as these evolution is not made. Since inheritance depends upon the continuity of material of the chromosomes of the germ cells, changes in adult structure or function can only be permanent when they follow a corresponding change in one or more chromosomes. These chromosomal changes may conceivably arise from within, or be impressed from without. Much of the modern investigation which has a bearing upon the method of evolution is concerned with the question whether the modifications of chromosomes are caused by internal or external agencies.

Two of the most striking cases of the origin of new heritable characteristics are those of the fruit fly *Drosophila* and the evening primrose (*Oenothera*). Scores of permanent changes in these organisms have appeared within the last decade. These new features have appeared in one individual among a hundred in the same bottle, or among a thousand in the same field. Environmental differences seem excluded in these cases. For, if one attributes these changes to invisible and unsuspected variations of the environment in circumscribed regions in a bottle or field, there is no need to appeal, as has usually been done, to the grosser elements like climate and medium, and

evolution is once more made wholly speculative. Inasmuch as the very instability of protoplasm, which accounts for the manifold metabolic processes that characterize living things, makes not only possible but highly probable alterations of the chromosomes which, in our present state of knowledge, must be regarded as of internal origin, the discovery of cases like those of the fruit fly and the evening primrose, in which environmental agency is apparently inadmissible, should leave no doubt that evolution *can* occur without reference to specific elements of the outer world.

What the actual method of producing changes in the chromosomes may be can only be conjectured. Morgan and his students have abundantly demonstrated that the continuous identity of chromosomes is, in at least one animal, an invention based on appearances; that the chromosomes of one individual are often not identical with those of its parents. The crossing over which they postulate is an even exchange of corresponding parts of two chromosomes. How this exchange is brought about, whether through the twisting of the chromosomes as the students of *Drosophila* have assumed, or because of the variability of the forces that hold the chromosomes together, as Goldschmidt (1917) suggests, is immaterial. If, occasionally, this exchange between the chromosomes is not equal, an occurrence that is not inconceivable, a chromosome might be produced unlike any that ever existed. If the germ cell containing such a chromosome were capable of producing a viable individual, to predict the probable nature of such an organism would be idle speculation.

Failure of the chromosomes to divide, and the passage of one or more of them bodily to one end of the spindle, would produce daughter cells with an unequal complement of hereditary material. Hyde (1916) has recently reported a case in *Drosophila* which is probably of this nature; the two X chromosomes appear to have remained undivided, going to opposite daughter cells, resulting in

the production of right and left eyes of different sex-linked colors. If, among the descendants of such unusual cells, germ cells should be produced, a new type of organism should result from their development. Although Babcock and Lloyd (1917) reject somatic segregation because one supposed case of that phenomenon reported from the Oregon Agricultural Experiment Station proved, in their opinion, to be something else, there is still some evidence, like that of Hyde's, that such unequal divisions do occur. If they occur in the line of the germ cells, new modifications may thereby be produced in the next generation.

Failure of the maternal and paternal chromosomes to separate in the reduction division of maturation, a phenomenon discovered in *Drosophila* by Bridges (1913) and named by him non-disjunction, may also be the cause of occasional evolutionary changes.

The foregoing chromosomal irregularities, which have the appearance of being mechanical rather than chemical phenomena, are not, however, necessarily the instruments with which permanent changes of organization are wrought. Probably they are not the usual ones. They have been mentioned first because there is evidence that such changes are occurring now. Minute changes far below the present limits of visibility are as conceivable, and in my opinion quite as probable, as the grosser ones named. In what these changes occur no one knows, for no one knows the nature of the hereditary elements. Suggestions involving enzymes and side chains have been made. These are only conjectures, but they reveal a belief that the phenomena of inheritance are chemical phenomena. If we believe that heredity is dependent upon chemical processes, there seems to me no escape from the assumption that evolution is first of all a chemical change. What the cause of these changes may be is another question; but if changes in considerable fragments of chromosomes, or even in whole chromosomes, can occur as a result of agencies within the organism, as

is plainly the case in *Drosophila* to-day, there is no reason to deny that the invisible modifications of chromosomes, if such occur, are likewise of internal origin.

In suggesting possible sources of internal change resulting in evolution I am not blind to the fact that an ultimate explanation of the method of evolution is not thereby offered. The chemical processes which cause these phenomena, while they are distinctly within the field of the geneticist, are not within his knowledge. If there were any prospect that an ultimate solution of the problem of the causes of germinal changes could be offered at the present time, invitation to participate in this discussion should have been extended to a physiologist; for it is from him that the eventual explanation of these internal changes must come.

In this account of possible ways in which changes in the chromosomes of germ cells arise, I have not forgotten that it is conceivable that the changes are forced by external agents. There are, indeed, biologists who regularly attribute such changes to environment. The paleontologists not infrequently seem to regard evolution as ordinarily so caused. But with a few exceptions, those who hold these views are not experimentalists. They are not the biologists who are engaged in studying present phenomena. They reason from results to cause. Out of the conceivable causes they have picked on one which has a chance of being the right one, but only a chance. I venture to suggest that the theory of internal origin of modifications will account for all paleontological, morphological, and geographical phenomena, and accord with all evidence from those fields, quite as well as the environmental theory.

Among the experimentalists, it is to be admitted, there are a few who occasionally proclaim the discovery of a modification produced by the environment and subsequently inherited. By one or two, not possessed of the still small voice, these proclamations are made repeatedly. Sometimes the effect of the environment is admit-

tedly directly upon the germ cells, and the results are not usually challenged. In other cases it is claimed to be only upon the soma, which then modifies the germ cells. These latter claims, however, meet with singular indifference or even distrust on the part of other biologists. Vulnerable places are too easily found, such as the lack of adequate controls. Sometimes the environmental evolutionist is charged with unwillingness or inability to show his hand when pressed for further information. Furthermore, it may seem strange that in a world of biologists, all anxious to solve the problem of the method of evolution, and all so far as I am aware willing that that method should be anything whatever, all of the important supposed cases of permanent modification caused by environment should be advanced by a handful of investigators.

To conclude: We have affirmed our adherence to the principle that evolution in past time is to be explained by phenomena that occur to-day. No processes that do not occur in living things now may be assumed to have occurred in living things formerly, unless there is plain evidence that events not explainable in terms of modern metabolism once occurred. Applying this principle only to the origin of modifications, not to their preservation, we have shown that animals are evolving now through agencies within themselves, independent of the environment. Whether environment also produces permanent modifications is questionable, with the burden of proof still resting upon those who hold that it does. All of the known steps of evolution *may* be explained as originating from within the animals' organization. There is no necessity of appealing to any other mode of origin, except, perhaps, to satisfy a certain type of imagination. In view of these considerations, it seems not illogical to me to suspect that evolution, at least among all but the very low animals and plants, is usually if not always initiated by a chemical change, either directly or indirectly produced, in the chromosomes of the germ cells; that these

changes are inherited because they result from changes in the chromosomes, and for no other reason; that such changes are usually, if not always, independent of the environment; that such changes produce unpredictable changes in adult structure or function; and that these changes have no reference to the usefulness of the change in the environment in which the animal exists or in any other environment.

If one desired to go beyond the first steps of evolution, and discuss the factors that determine the course of evolution by effecting the survival or destruction of such new forms, it would not be difficult to maintain that survival is much less dependent upon fitness than is commonly supposed, and that natural selection probably operates only to eliminate the most unfit. But such a proposition necessarily involves much speculation, with comparatively little information regarding present day phenomena to serve as guide. I shall content myself, therefore, with the above categorical statement of views regarding the origin of permanent modifications, and allow my colleagues to begin the sifting and testing operation which is theirs to perform.

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## SHORTER ARTICLES AND DISCUSSION

### AN INTRINSIC DIFFICULTY FOR THE VARIABLE FORCE HYPOTHESIS OF CROSSING OVER

THE assumption of a "variable specific force," made by Goldschmidt,<sup>1</sup> may seem to account for the frequency of the crossovers occurring in a given simple case of linkage; but when this explanation is extended to the results which such crossovers give when bred, it creates a difficulty of the same type and magnitude as the original problem of crossing over, for which, therefore, it is not a satisfactory solution.

Briefly put, the explanation advanced by Goldschmidt assumes that the genes are carried by the chromosomes, and that each gene is incorporated in its characteristic locus by virtue of a force residing in the chromosome and possessing properties specifically related to the properties of the genes of that locus. In the heterozygote  $Gg$  (see accompanying figure, line 1), the two forces  $F_g$  and  $F_g$  residing in the homologous chromosomes C and C' possess not only a locus specificity but also an allelomorphic specificity corresponding to the allelomorphs G and g. When the chromosomes of the  $Gg$  heterozygote go into a resting stage, these forces  $F_g$  and  $F_g$  relax, so that the genes G and g become freed. When the chromosomes are reassembled preparatory to division these forces again come into play with the result that gene G is again incorporated into the chromosome in which  $F_g$  resides, while gene g is likewise reincorporated into the homologous chromosome characterized by the presence of  $F_g$ . In order that crossing over may occur, the allelomorphic specificities of forces  $F_g$  and  $F_g$  must, in the first place, be commensurable variables; i. e., forces  $F_g$  and  $F_g$  must vary in that property which constitutes their essential difference, and in such a manner that when all the values of force  $F_g$  are represented by a characteristic frequency distribution and likewise all the values of  $F_g$  are represented by a second specific distribution, these two distributions will have a common base (see diagram, line 1). In the second place, these two distributions must overlap on the common base line so that a value chosen from the lower range of one may

<sup>1</sup> Dr. R. Goldschmidt, "Crossing over ohne Chiasmatypie?" *Genetics*, 2: 82-95.

be of the same magnitude as a value taken from the upper range of the allelomorphic distribution, though *the two forces thus chosen are, of course, no more identical than are two of Johannsen's beans which are of the same size but belong to different pure lines.* It is then assumed that in those cells in which the values of  $F_G$  and  $F_g$  are equal, the chromosome carrying  $F_g$  should incorporate gene  $g$  as frequently as gene  $G$ , and in those cells in which their normal order of magnitude is inverted, the crossover incorporation should occur more frequently, depending on the amount of inversion. Let us assume that in a given case this overlap is of such a per cent. that one per cent. of the gametes are crossovers (%1 of the diagram).

Now, in order to present the crux of the matter, let us proceed with the analysis of the behavior of the crossovers produced in the above experiment (see diagram, line 2). Let us mate a cross-

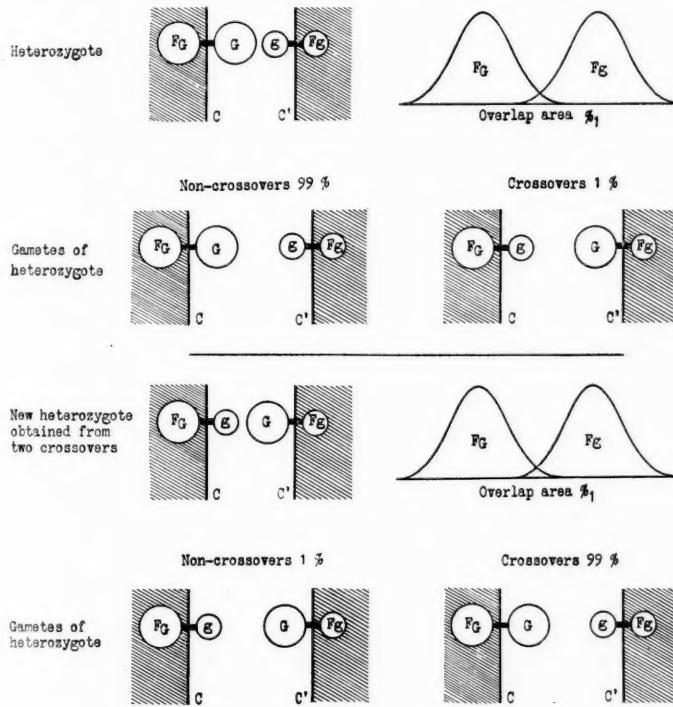


FIG. 1.

over individual in which gene G is held incorporated by force  $F_g$  with the converse crossover individual in which gene g is held incorporated by force  $F_g$  (see diagram, line 3). As soon as the chromosomes of the resulting heterozygote enter the proper resting stage the forces  $F_g$  and  $F_g$  relax, freeing the genes G and g. It must now be recalled that every value of force  $F_g$  is a member of a specific frequency distribution representing the entire behavior of  $F_g$ , and that any particular value of force  $F_g$  should give in succeeding generations the same result as every other value of  $F_g$ . Each value of  $F_g$ , whether chosen from the extreme upper range, the extreme lower range, or the mid-region, should give rise among its descendent cells to a series of variates which reproduce the original distribution  $F_g$  and no other. That is, the two distributions which describe the variates of  $F_g$  and of  $F_g$  in the cells of the new heterozygote, being specific, overlap in exactly the same fashion and to the same extent as did the distributions of the forces  $F_g$  and  $F_g$  in the original heterozygote (see diagram, line 3). Consequently, when the chromosomes are reassembled force  $F_g$  will, as before, incorporate gene G in 99 per cent. of cases and gene g in 1 per cent. of cases (see diagram, line 4). *But gene G entered the heterozygote as part of the chromosome possessing force  $F_g$ , hence the 99 per cent. of emerging offspring in which gene G is incorporated by the chromosome bearing  $F_g$  or gene g by the chromosome bearing  $F_g$  are crossovers.* As everyone acquainted with linkage knows, the crossovers given by the heterozygote from the mating of two crossovers are of the same frequency as in the original experiment. The intensities of coupling and of repulsion are *equal* and not complementary. Goldschmidt's machine which at the first revolution turned out a mere driblet of crossovers, should overwhelm the operator with a deluge of crossovers at the next turn of the crank. The whole explanation fails unless some added agency be devised to take over the duty which the specific allelomorphic forces abandon after the occurrence of crossing over.

The original problem was to secure the replacement of gene G in chromosome C by gene g, and at the same time the replacement of gene g in chromosome C' by gene G. Having assumed the machinery of specific variable forces to accomplish this interchange, we find that the products of the interchange are not stable, and furthermore they give a result the opposite of that demanded by the well known facts of linkage. In order that

gene G should be stably related to its new position in chromosome C' it must be held incorporated by force  $F_g$  and not by force  $F'_g$  as is the case. Added on to the original problem of the interchange of the genes is now the second and equally imposing problem of the interchange of the forces subsequent to the interchange of the genes. An actual bodily interchange of the forces seems impossible in view of the assumptions we have had to make as to their nature and action. The transformations would then have to be accomplished by some transmutation *in situ*. It is evident that no internal autonomous change short of a complete and absolute mutation of force  $F'_g$  in chromosome C into  $F_g$  and simultaneously of  $F_g$  in C' into  $F'_g$  would suffice. But we have no precedents for assuming such reciprocal mutations, and if we had, we could have sidetracked this whole machinery by applying this reciprocal transmutation idea to the genes and thereby solved the first problem in such a way that the second could not arise. Instead of localizing the cause of the reciprocal transformations of the forces in the forces themselves, one might transfer it to the genes; *i. e.*, one might endow the genes with the power of causing reciprocal transformations of the forces rather than empower the forces to transmute of their own accord. While this form of the transmutation idea carries something of an air of plausibility, it can not be taken as more than an attempt at formal escape from the difficulty—a lifting of one's self by one's boot straps that makes more demand on credulity than, for example, one would in assuming crossing over offhand as a specific property of genes which needs, as support, only such formal explanation.

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#### ON THE PROBABLE ERROR OF MENDELIAN CLASS FREQUENCIES

AN old friend of geneticists who dislike excessive calculation has recently been attacked by Pearl,<sup>1</sup> viz., the familiar formula,  $\sigma = \sqrt{npq}$  for the standard deviation of a Mendelian class frequency. He proposes to substitute a more refined but much more complicated method, originated by Pearson. In a Mendelian illustration he obtains a result which differs by over 40 per cent.

<sup>1</sup> Pearl, R., "The Probable Error of a Mendelian Class Frequency," AMERICAN NATURALIST, Vol. LI, pp. 144-156, 1917.

from the usual. This seems to indicate that the old method is wholly inadequate, but further examination shows that the difference is not due so much to method as to the fact that Pearl has calculated something with a different significance from the usual probable error. A cross of Mendelian heterozygotes (Blue Andalusian fowls) gave three classes of young in the numbers 14:33:11. Expectation is 14.5:29:14.5. Pearl assumes that a first sample of 58 has given exactly expectation and then calculates the quartile deviations for each class in a second sample of 58. The results are given as 3.13 for the heterozygous classes, 3.55 for the homozygotes which indicate an excellent fit of observation to expectation. By the usual method, if a first sample of 58 had given exactly 14.5 black chicks and nothing were known of any theoretical expectation, the probable error in a second sample of 58 is measured by the probable error of differences. The probable error of either sample as given by the formula  $.6745\sqrt{npq}$  is 2.22. The probable error of differences by the usual formula  $.6745\sqrt{\sigma_1^2 + \sigma_2^2}$  is 3.15. This does not differ appreciably from Pearl's quartile of 3.13. Neither of these methods, however, gives what we really wish to know, the closeness of fit to Mendelian expectation. We have a theoretical expectation which is not based merely on a particular sample of 58, but which should hold with increasing accuracy the larger the first sample taken. With an infinite first sample, the formula given by Pearl reduces to the usual one,  $.6745\sqrt{npq}$  giving a quartile of 2.22. This is less lenient to the discrepancy between expectation and observation than the first result, but the fit is still not bad. In a second illustration which is given, we do have two samples and no theoretical expectation suggested. The usual method of comparing samples of different sizes would be to find the standard deviation of differences on a percentage basis. The percentage standard deviation for a sample of  $n$  individuals is  $\sqrt{pq/n}$ , for a sample of  $m$  individuals is  $\sqrt{p'q'/m}$  and for differences is  $\sqrt{(pq/n) + (p'q'/m)}$ . The expected standard deviation of a sample of  $m$  individuals is, however,  $m\sqrt{(pq/n) + (pq/m)}$  if  $p$  and  $q$  are based merely on the first sample as in Pearl's illustration. The formula given by Pearl for the standard deviation rapidly approaches this form for large values of  $m$  and  $n$ . Following are the results given by the long method, by an approximation given by Pearl and by the usual one just cited.

	Long Method	Approximate Method	Usual Method
Median .....	83.53	83.95	83.71
Lower quartile.....	75.61	75.84	75.64
Upper quartile.....	91.82	92.06	91.79

The usual method gives substantially the same result as the long one and a better result than the approximate method. From the nature of experimental work, great refinement in statistical treatment is often a waste of effort, and without questioning the value of Dr. Pearl's suggestion in cases in which the greatest accuracy is warranted it appears that the simple formula is still adequate for most practical purposes.

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BUREAU OF ANIMAL INDUSTRY,

March 12, 1917

CHARACTERS INDICATIVE OF THE NUMBER OF  
SOMATIC CHROMOSOMES PRESENT IN  
ENOOTHERA MUTANTS AND  
HYBRIDS

THE pollen grains of 28-chromosome *Oenothera Lamarckiana gigas* de Vries were long ago shown (Lutz, '09)<sup>1</sup> to be characteristically 3+-lobed (chiefly 4-), instead of 3-lobed, as in *O. Lamarckiana* and other diploid forms. Gates has since contributed much to our knowledge of this subject. Recently Bartlett ('15)<sup>2</sup>, in discussing the 3+-lobed condition of the pollen of 28-chromosome *O. stenomeres* mut. *gigas*, stated that these 3+-lobed grains "are larger than the triangular grains of the type" (*O. stenomeres*). It may be added that the largest, best-appearing 3- of tetraploid forms in general, is larger than the typical, best-appearing 3- of diploid, and the largest, best-appearing 3+- of the former, larger than the typical, occasional 3+- of the latter. Smaller 3- and 3+-grains are found in the pollen of both, but they are rarely perfect-appearing, and it is doubtful whether slightly imperfect-appearing grains are capable of functioning. A careful examination of the adult characters of a form, together

<sup>1</sup> Notes on the first generation hybrids of *Oenothera lata*  $\times$  *O. gigas*, *Science*, N. S., 29: 263-267. Gates (Pollen development in hybrids of *Oenothera lata*  $\times$  *O. Lamarckiana*, *Bot. Gaz.*, XLIII, 81-115, Feb., 1907) had earlier observed 3+-lobed grains in the pollen of a triploid form.

<sup>2</sup> "The Mutations of *Oenothera stenomeres*," *Amer. Jour. Bot.*, 2: 100-109.

with a microscopical examination of the pollen of 10-15 buds from different parts of the plant will enable one to estimate its probable somatic chromosome number; this estimate becomes more trustworthy when one considers also (using *Lamarckiana* as the standard for comparison) the number of seeds produced per fruit by selfed flowers, the percentage of seeds which germinate, and the hereditary behavior of the plant.

### I

All parts, or most parts, slender; pollen consisting of small, 3-lobed grains with an occasional  $3 + -$ . Plant will probably be found to be diploid, or approximately so, having 14 or 15, possibly 16, chromosomes.

A. Abundance of pollen, 50 per cent. or more of the grains good-appearing. Abundance of seed secured from selfed flowers, germinating about as for *O. Lamarckiana* when sown in soil; all offspring, with the exception of a few mutants, duplicating the characters of the parent. Plant will probably have 14 chromosomes.

B. (a) Anthers barren as for *Lamarckiana lata*, or (b) variable amounts of pollen produced in different buds of same plant; entirely absent in some, present in small quantities or in moderate amounts in others. In best buds, imperfect-appearing grains considerably in excess of good-appearing; in others, greatly in excess of them and in still others, very few good-appearing grains. Fewer seeds obtained from selfed flowers than from an equal number of selfed *Lamarckiana* flowers; lower percentage capable of germinating, when sown in soil, than is usual for *Lamarckiana* seeds. Plant will probably have 15, possibly  $14^{+1}$ , or even 16, chromosomes.

### II

Plants intermediate between *O. Lamarckiana* and *O. Lamarckiana gigas* in stoutness of all parts; pollen absent, present in small quantities, or in moderate amounts, much as for I B (a) and (b). Pollen containing a mixture of 3- and  $3 + -$  lobed grains, the former exceeding the latter in number. Largest grains larger than typical, best-appearing grains of diploid forms; relatively few of the grains good-appearing, even in best buds. Selfed flowers produce no seeds or very few; when seeds are sown in soil, very small percentage germinate. Plant probably triploid, or approximately so; probably 21-chromosome form if offspring of selfed 14-chromosome plant, or of  $14 \times 28$ ; possibly 20 or 22 chromosomes. If produced by a selfed 15-chromosome form, or by  $15 \times 28$ , the chances of its having 22 are greatly enhanced.

### III

All parts stouter than for triploid forms; pollen grains characteristically  $3 + -$  lobed, with relatively few 3-lobed (typical grain 4-). Largest and best-appearing 3- and  $3 + -$  lobed grains larger than typ-

ideal, best-appearing 3- and occasional 3+lobed grains of diploid forms; 40 per cent, or fewer, good-appearing. Moderate amount of seed obtained from selfed flowers. Seeds large, germinate quickly. Plant will probably have 28 chromosomes, particularly if an offspring of a 14- or a 28-chromosome form, selfed, or of a 14 x 28. If the product of 15 x 28, it may have 28, 29, or even 30, chromosomes, I. Forms which are approximately, but not precisely, tetraploid, may be wholly male-sterile.

These statements are not intended to imply that all diploid, triploid and tetraploid forms have the characters enumerated above, but merely that forms displaying certain pollen conditions and vegetative characters will *probably* (by no means certainly) have the number of chromosomes specified.

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#### ON THE PERIODIC SHOREWARD MIGRATIONS OF TROPICAL NUDIBRANCHS<sup>1</sup>

MANY northern gastropods, including nudibranchs, are well known to exhibit the habit of congregating in shallow water along the shore at their time of breeding. This has been commonly interpreted as the result of migration from deeper water at the approach of the egg-laying season. Certain species, at any event, are from time to time found in great quantity at shore stations which they do not frequent at other periods, and field observations have apparently established beyond a doubt that this inshore appearance is closely connected with mating and oviposition. The migration into shallow water, or other means which accomplishes the shallow-water flocking in these cases, may be regarded as a device which insures the concentration of individuals within a relatively small area, thus tending to make more certain the chances of pairing in a large number of instances, as well as a method of determining favorable conditions for larval development.

Collectors of nudibranchs who have worked in tropical waters have also reported cases which at first sight seem to afford additional examples of the coincidence of the spawning period with appearance in great numbers in the littoral zone (*e. g.*, Crossland, quoted by Eliot, 1904, p. 87). While engaged in working along the shore during a period of some days or weeks, it is

<sup>1</sup> Contributions from the Bermuda Biological Station for Research, No. 59.

noticed that a certain species of nudibranch, until then found sparsely, if at all, suddenly begins to occur in abundance. It is also observed that at this time these nudibranchs are depositing eggs in the field, or that they pair readily and lay egg strings when kept in aquaria. The inference which has been drawn in such cases, namely, that the appearance in shoal water is in some way intimately related to the mating process, seems legitimate enough.

But I have observed at Bermuda certain facts regarding the normal migrations of a member of the typically tropical genus *Chromodoris* which, it seems to me, cast considerable doubt on the theory that this species, *C. zebra* Heilprin, moves into shallow water for the purposes of mating and egg deposition. The facts in this case, so far as they have been observed, are briefly as follows:

It was necessary to obtain considerable numbers of *C. zebra* for use in experimental work (Crozier, 1916<sup>a</sup>, 1916<sup>b</sup>); consequently collections were made at short intervals (every day during some months) over the period from August, 1915, to October, 1916. I had had occasion, also, to note the occurrence of this species in the summers of 1913 and 1914. In June, and during the early part of July, *Chromodoris* was found in great abundance upon the "eel grass" in certain tidal "creeks" (Fairyland Creek, Millbrook Creek). Subsequently, in the last two weeks of July and in August, they became very scarce in such places, although a few could almost always be discovered by careful searching. At other times of the year a supply of the animals was obtained on hard, open bottoms in somewhat deeper water (1 to 2 fathoms, at low tide), in places where, I am certain, they would never have been seen during ordinary shore collecting. Occasionally, however, as was noted particularly in December, 1915, *Chromodoris* was abundant along the rocky shores of smaller islands, ranging well up to low-water level.

It seems clear enough that in *Chromodoris zebra* there undoubtedly does occur from time to time a movement of numbers of individuals toward the shore. But there are several facts which sharply contradict the view that this migration is connected with reproduction. The nudibranchs pair in the laboratory and lay strings of fertile eggs at all seasons of the year (cf. also Smallwood, 1910), and not merely at the times when

they are abundant near low-water level. Moreover, I have obtained the egg masses in dredgings at every season of the year; hence we may regard the fact of egg laying at all seasons under laboratory conditions as of significance in this connection. The eggs, which are quite characteristic in appearance, and hence easily identified, have been collected in depths of eight fathoms and more. Large individuals of *C. zebra* are likewise not uncommon at these depths; in fact, the first ones to be described were dredged from ten fathoms in Harrington Sound (Heilprin, 1889, p. 187). A further point of considerable significance is found in the fact that these nudibranchs, unlike *Elysia* and certain other species, do not appear to deposit any egg masses upon the "eel grass" on which the animals occur in such great numbers throughout the early summer. The egg strings found in the field are invariably attached to rocks, or to the shells of *Arca noe*, the "mussel" with which the adults are frequently associated. The gelatinous egg-ribbons (cf. Smallwood, 1910) are quite large, measuring usually 120 to 150 mm. long by 15 to 17 mm. broad, and are much too heavy to be supported by a blade of "eel grass," as can readily be determined by trial. It is only rarely that an egg mass has been obtained in shore collecting.

The migration of *C. zebra* into shallow water cannot, then, be directly connected with reproductive activities. Since, in the laboratory at least, they deposit eggs usually within twenty-four hours after pairing, it does not seem to me probable that these nudibranchs pair to any great extent during the time which they appear to spend in the tidal "creeks"—no eggs, as stated, having been collected from among the "eel grass," nor were any ever obtained on the muddy bottoms of these "creeks." *Chromodoris* seems to require a firm, hard substratum for the attachment of its egg-ribbon. If individuals obtained in quite shallow situations are kept singly in aquaria they sometimes deposit after several days fragments of egg-jelly containing several dozen unfertilized eggs, while they almost invariably pair readily when given the opportunity. Nevertheless, it should be stated that the nudibranchs usually do not occur singly, two or three being commonly found within a space of several square meters even when the total number of individuals in a given area is small; and I am well aware that laboratory findings with regard to breeding habits are liable to be misleading. The established

fact of egg production throughout the year in deep water is, however, good evidence that the periodic (or intermittent) abundance of this nudibranch in shoal situations can have little if any relation to oviposition.

It might at first be suspected that the periodic shoreward movement represents the phylogenetic persistence of a well-defined habit possessed by not distantly related northern species. From this standpoint, reproductive functions in *C. zebra* might be conceived of as having become dissociated from the habit of migratory periodicity, since in warmer seas, where the seasonal alteration in physical conditions is reduced to a minimum, it is well known (cf., for example, Semper, 1881, p. 135) that many forms have no specially restricted time for breeding. However attractive such a speculation may appear, it is eminently more satisfactory to regard these periodic littoral appearances of tropical nudibranchs as being controlled by definite physical influences in each individual case. Such directing causes would not necessarily be always the same for each periodic occurrence of the animals in shallow water. Although shoreward migration and egg laying are closely connected in northern forms, it is still probable that physical circumstances in the sea immediately control the migrations even in this instance also.

I have purposely refrained until now from discussing certain minor fluctuations in the littoral abundance of *Chromodoris* which are, nevertheless, important in connection with the idea that the supposed "migration" at certain times into very shallow water is, after all, only the unrestricted expression of a tendency to upward movement—negative geotropism. It has been mentioned that during the greater part of the year *Chromodoris* was collected in 1 to 2 fathoms. But after storms of some severity they were to be had only in much deeper water. The nudibranchs undoubtedly move into deep, quiet places when the surface is greatly disturbed. Just what their behavior is under these circumstances can not be stated from direct observation, for obvious reasons; and for several days, or even for a week after a severe blow, the water in the sounds and bays remains so roily that it is impossible to see the bottom. But I have frequently observed individuals creeping up from deep water after the sea has become quiet and transparent. As regards the bearing of these facts upon the major flocking into the littoral zone, which occurs in early summer, it is to be noted that the mere

continuance of quiet, still weather is not enough to determine the abundance of *Chromodoris* in the tidal "creeks," since they disappear for the most part before the calm summer season is half over. The occurrence of individuals in deep water, together with field observations of specimens which were engaged in creeping downward on the sloping sides of rocks and reefs, leads me to doubt very much that any form of geotropic irritability exerts a preponderant control over the normal behavior of these animals. My observations strongly suggest, however, that there does occur to some extent (in appropriate places) a diurnal vertical movement of *Chromodoris*, which is directly determined by the positive phototropism of these nudibranchs.<sup>2</sup>

Specimens of the species known as *Chromodoris roseapicta* Verrill (there is some doubt that it is really a *Chromodoris*) have been found in littoral locations, only in the summer time, but this type is not sufficiently abundant to make possible a testing out of ideas concerning its migratory movements.

The point which I wish to emphasize most is the uncertain nature of conclusions having reference to the normal behavior of animals inhabiting the warmer seas on the basis of comparisons with superficial features of the movements of their relatives in colder waters. In the case of *Chromodoris zebra*, it seems to me definitely established that the periodic flocking of individuals into very shallow water has no immediate connection with reproduction.

On Jan. 10th, 1917, I found that *C. zebra* was crowding in great numbers into the entrance of Fairyland Creek. During the next few days they became very abundant indeed, so that on one occasion 230 of them were picked up in less than an hour's collecting. On Jan. 12 I began to find egg masses attached to certain sponges, matted algae, mangrove roots, and sundry mooring stakes in the "creek." I had not before found any in this place, as stated above. The nudibranchs were observed in copulation, and great numbers of egg-masses were found. The attachment of the egg-masses was most frequently to some firm object. Within the week Jan. 10-17 they began to disappear, and after a fairly severe storm which came at that time very few were obtainable in the "creek." This occurrence seems to form a good

<sup>2</sup> I am anticipating here the statement of certain facts regarding the responses of *C. zebra* which were established in this laboratory several years ago by Dr. L. B. Arey (*cf.* also Crozier, 1916<sup>c</sup>).

instance of shoreward movement coupled with reproductive activity, but the fact remains that the nudibranchs do breed abundantly at other times and in much deeper water.

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AGAR'S ISLAND, BERMUDA.

W. J. CROZIER

## NOTES AND LITERATURE

### DEAN AND EASTMAN'S BIBLIOGRAPHY OF FISHES

In order that the production and diffusion of knowledge may but be promoted, knowledge gained must be published in some permanent form. But when the publications become numerous and scattered throughout many journals, and in various languages, it becomes at length difficult, or even impossible, for any human being to retain in mind all that others have discovered and written. The literature must be organized in such a way that the seeker after knowledge and the producer of knowledge may be enabled to determine easily what has been published on any particular subject. Hence the need for bibliographies and bibliographies of bibliographies, for the *Zoological Record*, and the International Catalogue of Science. Hence the justification, the necessity, for Dean and Eastman's Bibliography of Fishes. Dean tells us in the preface that in this work there are listed more than 40,000 titles. How small a number of these could any man command were it not for some such collection!

One volume only of the work has yet appeared. This is a book of 718 octavo pages of small print; and this is occupied simply by the authors' titles of papers alphabetically arranged. And only those authors have been reached whose names begin with the letters A-K. A second volume is to follow which is to include the others. The time, the patience and the labor which the accumulation of such a list demands may be surmised by the reader of the preface; it can only be realized by one who has tried his hand at something of the kind himself.

As the work will then stand, the student of fishes can determine readily all the papers that any author, as Agassiz or Baird for example, has written; or he can glance over all the 40,000 titles and pick out those which seem to have a bearing on his subject. To obviate the latter necessity, a third volume is to follow which is to be an index to the preceding volumes. In the two volumes of authors' titles each paper is followed by the year of publication and a serial number, as "Jordan, 1891, 4"; and in the index each paper is to be referred to briefly by the author's name, the year and the serial number. Economy of labor and

expense is thus effected. The index will certainly be classified in such a way as to make it reasonably easy to arrive at the papers desired. In estimating, therefore, the work that Dr. Dean and his editor and assistants have done we must consider not only the collection and preparation of the titles, but likewise the analysis of these papers and the reording of the contents under their proper heads.

There is a need for more yet to be done. The author tells us that the index does not include detailed references to species, genera, or even, in many cases, families of fishes. "This would entail many years' additional listing, but should unquestionably next be done." The busy student may want to know what has been written on the Centrarchidae, or the genus *Lepisosteus*, or the rainbow trout; and he ought to find all of the papers recorded under each head. May the good men who have worked on this Bibliography of Fishes retain their powers and live long enough to accomplish the work.

However, it will be open to any one to go through those 40,000 books and papers and cull out the things bearing on the subject he has chosen and to publish a little bibliography of his own.

The present writer has not undertaken to discover omissions of papers or errors in quoting them. Certainly omissions and errors occur, as in any human production; but doubtless all possible care has been taken to avoid them. Two omissions have incidentally been brought to notice. The first of these is a paper by Eigenmann on a fossil species of *Sebastodes*, in *Zoe*, Volume I, 1890, page 17; although another paper cited ends with page 15. The other paper omitted is B. K. Emerson's "Geology of Old Hampshire County, Massachusetts," in which there is a list of the Triassic fishes found in the state mentioned. Dr. Dean must have had a record of this paper.<sup>1</sup> A paper by E. W. Claypole<sup>2</sup> is quoted from the *American Geologist*, Volume XXIX, p. 44; but the paper is not found as cited; nor elsewhere, so far as the present writer knows.

OLIVER P. HAY

<sup>1</sup> *Science*, Vol. XVI, 1902, p. 701.

<sup>2</sup> Claypole, 1890, 1.

